

Article

Plasma-Activated Water Improves Seed Germination and Seedling Vigor in Hot Pepper (*Capsicum annuum* L.) via Redox-Mediated Hormetic Regulation

Chanachai Natthaaek ¹, Nopporn Poolyarat ², Bhornchai Harakotr ¹, Yaowapha Jirakiattikul ¹ and Panumart Rithichai ^{1,*}

¹ Department of Agricultural Technology, Faculty of Science and Technology, Thammasat University, Pathum Thani 12120, Thailand; chanachainattaek@gmail.com (C.N.); harakotr@tu.ac.th (B.H.); yjirakia@tu.ac.th (Y.J.)

² Center of Advanced Nuclear Technology, Thailand Institute of Nuclear Technology (Public Organization), Nakhon Nayok 26120, Thailand; noppornp@tint.or.th

* Correspondence: panumart@tu.ac.th

Highlights

What are the main findings?

- PAW30 significantly enhanced seed germination, seedling vigor, and reserve mobilization in hot pepper seeds.
- PAW-induced responses were associated with changes in redox status, antioxidant regulation, and reserve mobilization.
- Seed responses exhibited a dose-dependent hormetic pattern, with excessive PAW activation increasing oxidative stress indicators and reducing seed vigor.

What is the implications of the main finding?

- PAW represents a promising eco-friendly priming strategy for improving seed performance in hot pepper.
- Understanding PAW-mediated redox regulation may facilitate the optimization of seed priming technologies for horticultural crops.

Abstract

Plasma-activated water (PAW) is an eco-friendly seed priming agent, yet its efficacy depends on plasma activation conditions that remain insufficiently defined. This study characterized the physicochemical properties of PAW generated at different activation times (15–120 s) and evaluated their effects on germination and biochemical responses of hot pepper (*Capsicum annuum* L.) seeds, in comparison with non-primed and distilled water-primed controls. Increasing plasma activation time elevated electrical conductivity and reactive oxygen and nitrogen species (RONS; e.g., H₂O₂ and NO₃[−]), while lowering pH, with NO₂[−] peaking at intermediate durations. PAW priming induced a dose-dependent hormetic response, with PAW30 exhibiting the highest germination and seedling vigor. This improvement was associated with enhanced antioxidant enzyme activities, reduced oxidative damage, and increased α -amylase activity and soluble sugar accumulation, indicating improved metabolic activation. In contrast, longer activation times, particularly PAW120, increased oxidative stress markers and reduced the magnitude of germination and seedling vigor improvement relative to PAW30. Principal component analysis (PCA) further supported coordinated relationships among PAW properties, biochemical responses, and germination traits. Collectively, the results support a redox-associated hor-

Academic Editor: Ertan Yildirim

Received: 22 May 2026

Revised: 26 June 2026

Accepted: 29 June 2026

Published: 1 July 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

metic response to PAW priming and identify PAW30 as the most effective activation condition for improving germination and early seedling growth in hot pepper.

Keywords: antioxidant enzymes; *Capsicum annuum*; hormesis; reactive oxygen and nitrogen species; redox regulation; seed priming; plasma-activated water

1. Introduction

Hot pepper (*Capsicum annuum* L.) belongs to the Solanaceae family and is an economically important horticultural crop widely cultivated in tropical and subtropical regions. In addition to its culinary value, hot pepper contains capsaicin, a bioactive compound with diverse pharmacological properties, including anti-inflammatory, analgesic, and potential anticancer activities [1–3]. The increasing demand for high-quality hot pepper production highlights the importance of efficient crop establishment. In particular, seed germination and early seedling development are critical stages that strongly influence seedling uniformity, transplant quality, and subsequent crop performance. However, germination of hot pepper seeds is often characterized by low uniformity and sensitivity to environmental conditions, leading to poor seedling establishment and reduced crop performance in commercial systems [4,5].

Seed priming has been widely adopted as an effective pre-sowing strategy. This technique involves controlled hydration of seeds to initiate metabolic processes without allowing radicle emergence [6,7], thereby improving germination rate, uniformity, and early seedling growth. Among emerging priming approaches, plasma-activated water (PAW) has attracted considerable attention as an environmentally friendly technology. It is produced by exposing distilled water to cold plasma generated either above or below the liquid surface [8,9]. This process leads to the formation of reactive oxygen and nitrogen species (RONS), such as hydrogen peroxide (H_2O_2), nitrite (NO_2^-), and nitrate (NO_3^-), which alter the physicochemical properties of water [10,11]. These reactive species play a dual role in plant systems. At moderate levels, reactive oxygen species (ROS), particularly H_2O_2 , function as signaling molecules that regulate germination-related processes. These include water uptake, enzyme activation, and antioxidant defense, thereby promoting faster and more uniform germination [12,13]. Similarly, reactive nitrogen species (RNS), especially nitric oxide (NO), contribute to the regulation of seed germination and early plant growth [14,15]. However, excessive accumulation of RONS disrupts cellular homeostasis, leading to oxidative stress, biochemical alterations, and lipid peroxidation [16]. Therefore, the biological effects of reactive species depend largely on the cellular redox environment and the balance between their production and detoxification. Seed germination is strongly regulated by the cellular redox environment, which reflects the balance between ROS production and antioxidant defenses [17,18]. According to the oxidative window concept, successful germination requires an optimal level of ROS; insufficient ROS may limit the activation of germination-related processes, whereas excessive ROS can cause oxidative damage and impair seed viability [6,17,19,20]. Moderate redox signaling promotes reserve mobilization, cell wall loosening, hormonal regulation, and embryo growth, whereas excessive oxidation disrupts membrane integrity and metabolic activity [6,17,18,20,21]. Consequently, seed performance is closely associated with the maintenance of redox homeostasis, and treatments that modify RONS levels may either stimulate or inhibit germination depending on the magnitude of the redox shift [18–20]. This concept is particularly relevant to PAW, which acts primarily through the delivery of plasma-generated RONS to seeds.

The biological effects of PAW are closely associated with its physicochemical properties, which are strongly influenced by plasma activation parameters, particularly exposure time. Therefore, characterization of PAW physicochemical properties is essential to understand its subsequent effects on seed biochemical responses and germination performance. Increasing activation duration generally leads to higher accumulation of RONS, reduced pH, and increased electrical conductivity (EC) [22,23]. These changes modulate plant responses in a dose-dependent manner, where optimal activation enhances germination and growth, whereas excessive activation may diminish the beneficial effects and induce physiological stress [24,25]. PAW has been shown to improve germination and early seedling growth in crops such as soybean and wheat [26], and to promote growth in vegetable species including tomato and cabbage [27]. More recently, diluted PAW was reported to enhance germination and sprout growth in several sprout crops through redox-mediated physiological regulation and autophagy-related responses [28]. However, plant responses to PAW vary among species and treatment conditions, suggesting that its effects are context-dependent. Moreover, many PAW seed studies have focused primarily on germination outcomes and early seedling growth/development [23,24,26,29–31], whereas fewer studies have integrated PAW physicochemical properties with seed redox status, antioxidant responses, reserve mobilization, and multivariate germination performance, although some studies have examined selected physiological and biochemical responses in seedlings [32]. In particular, the influence of plasma activation duration on PAW properties and their relationships with seed biochemical responses and germination performance has not been systematically investigated in hot pepper. This study hypothesizes that PAW induces a dose-dependent response in hot pepper seeds, in which optimal levels of reactive species enhance antioxidant activity and promote germination, whereas excessive activation shifts the response toward oxidative stress and reduced seed vigor. Therefore, this study aims to characterize the physicochemical properties of PAW generated at different plasma activation times and to evaluate its effects on biochemical responses and germination performance of hot pepper seeds primed with PAW. Principal component analysis (PCA) was further used to examine coordinated relationships among PAW physicochemical properties, seed biochemical responses, and germination-related traits.

2. Materials and Methods

2.1. Plant Material

F₁ hybrid hot pepper seeds, cultivar Spark, were purchased from Tiger Seed Co., Ltd. (Nonthaburi, Thailand) as a commercial seed lot. According to the supplier, the seeds were harvested in April 2024 from plants cultivated in Chiang Mai Province, Thailand. The initial germination rate was 90%, and the initial seed moisture content was 6.76%.

2.2. Preparation of Plasma-Activated Water (PAW)

Distilled water (100 mL) was placed in a beaker and activated using a direct arc plasma device (Figure 1). The system consisted of four tungsten needles (150 mm in length and 1.7 mm in diameter) arranged in a rectangular array with center-to-center electrode spacings of 9.7 and 8.3 mm. The electrodes were enclosed within a nylon outer casing and connected to a high-voltage flyback transformer (15.8 kV peak-to-peak, 10 kHz DC pulsed power). An external rectangular metal electrode served as the ground electrode. Plasma was generated under ambient air conditions without an external gas supply. During activation, the electrode tip was positioned 3 cm above the water surface, and the solution was continuously stirred using a magnetic stirrer (MS500, INTLLAB, Shenzhen Jiashi Technology Co., Ltd., Shenzhen, China). Plasma exposure was applied for 15 s (PAW15),

30 s (PAW30), 60 s (PAW60), and 120 s (PAW120). Freshly generated PAW was transferred to sealed containers and stored at 30 ± 2 °C prior to use. Seed priming treatments were initiated within 1 h after PAW generation. Physicochemical properties of PAW, including pH, EC, H_2O_2 , NO_2^- , and NO_3^- concentrations, were also determined within 1 h after generation to minimize changes in RONS during storage. All PAW treatments were prepared under the same water volume, electrode distance, stirring condition, and storage duration to ensure consistency among activation-time treatments.

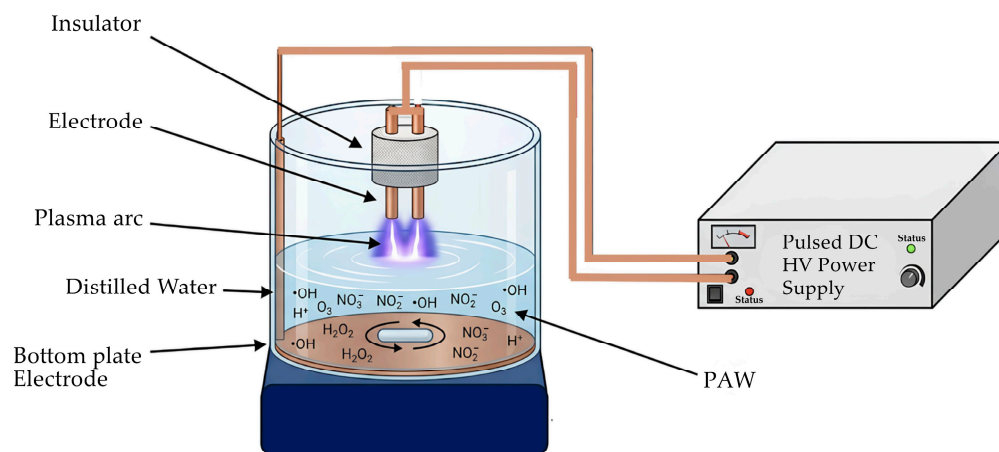


Figure 1. Schematic diagram of the direct arc plasma system used for plasma-activated water (PAW) generation.

2.3. Physicochemical Properties of PAW Determination

The EC and pH of PAW were measured using an EC meter (Hanna HI98311, Hanna Instruments, Nuşfalău, Romania) and a pH meter (Lab855, SI Analytics GmbH, Mainz, Germany), respectively.

The hydrogen peroxide (H_2O_2) concentration in PAW was determined according to the method described by Hou et al. [33] with slight modifications. Briefly, 100 μL of PAW was mixed with 500 μL of 0.2% (*w/v*) o-phenylenediamine (OPD) and incubated at 45 °C for 10 min. Subsequently, 250 μL of 10 N sulfuric acid was added, and the mixture was further incubated at 45 °C for 2 h. The absorbance was measured using a 96-well microplate reader (EnVision™ multimode plate reader, Revvity Inc., Waltham, MA, USA) at 490 nm. The H_2O_2 concentration was expressed as mg L^{-1} .

The concentrations of nitrite (NO_2^-) and nitrate (NO_3^-) were determined using a colorimetric method following Chuea-uan et al. [34], with slight modifications. NO_2^- was analyzed using a nitrite test kit (HI3873-0), while NO_3^- was measured using a nitrate test kit (HI3874-0), both from Hanna Instruments, Smithfield, RI, USA. The NO_2^- reagent contained potassium disulfate ($\text{K}_2\text{S}_2\text{O}_8$), whereas the NO_3^- reagent consisted of cadmium, potassium disulfate, and sulfanilic acid. For analysis, 10 mL of PAW was mixed with the respective reagents according to the manufacturer's instructions and shaken for 15 s. After color development, absorbance was measured using a microplate reader at 507 nm (NO_2^-) and 372 nm (NO_3^-). Quantification was based on external calibration curves (0–10 mg L^{-1} ; $R^2 = 0.9999$ for NO_2^- and 0.9995 for NO_3^-). Distilled water served as the reagent blank, and each sample was analyzed in four technical replicates. Results were expressed as mg L^{-1} .

2.4. Experimental Design and Seed Priming Procedure

The experiment was conducted in a completely randomized design (CRD) with six treatments and four replicates. The treatments comprised non-primed seeds (NP), seeds primed with distilled water (DIW), and seeds primed with PAW generated at activation

times of 15, 30, 60, and 120 s (designated as PAW15, PAW30, PAW60, and PAW120, respectively).

For the priming procedure, 2 g of hot pepper seeds were soaked in 20 mL of PAW (PAW15–PAW120) or DIW under dark conditions at 20 ± 2 °C for 24 h, followed by an additional 24 h incubation in a moist chamber under the same conditions to ensure uniform imbibition. The priming duration was selected based on preliminary observations to ensure sufficient imbibition without radicle emergence and to provide uniform hydration among seeds prior to germination testing. After incubation, the seeds were rinsed with distilled water and dried at 30 ± 2 °C. During drying, seed weight was periodically monitored, and drying was terminated when the seed weight returned to approximately the initial pre-priming weight, indicating restoration of the original seed moisture level.

The primed seeds were subsequently used for germination tests. For biochemical analyses, the dried-back seeds were ground into a fine powder under liquid nitrogen and stored at -20 °C until further analysis. All biochemical measurements were expressed relative to the weight of the dried-back seed material used for extraction (g^{-1} seed weight).

2.5. Seed Germination and Vigor Assessment

Seeds were germinated using the top-of-paper (TP) method in plastic boxes ($15 \times 10 \times 6$ cm) with four replicates of 50 seeds each. Seeds were placed on moist sterilized paper and incubated at 20 ± 2 °C under a 12 h light/12 h dark photoperiod, 70% relative humidity, and photosynthetically active radiation (PAR) of $8.6 \mu\text{mol m}^{-2} \text{s}^{-1}$. Moisture was maintained daily by adding 10 mL of distilled water to each box. Germination percentage (GP) was determined from the number of normal seedlings at 7 and 14 days after sowing according to ISTA [35]. Germination counts were recorded daily for 14 days to calculate mean germination time (MGT) and germination index (GI) following the methods of Geneve [36] and Maguire [37], respectively.

For accelerated aging, seeds were incubated at 41 °C and 100% relative humidity for 48 h, following Bissoli et al. [38], and germination percentage after accelerated aging (GAA) was assessed as described above.

Seedling growth was evaluated using the between-paper (BP) method with four replicates of 25 seeds each. After 14 days, shoot length, root length, and fresh and dry weights were measured. Dry weight was determined after oven-drying at 70 °C for 72 h.

2.6. Determination of Biochemical Responses

Hydrogen peroxide (H_2O_2) content was determined according to Billah et al. [39] with minor modifications. A 0.5 g sample was homogenized in 0.1% (*w/v*) trichloroacetic acid (TCA) and centrifuged at $10,000 \times g$ for 15 min. The supernatant was reacted with potassium iodide (KI) in 10 mM phosphate buffer (pH 7.0) and incubated in the dark for 1 h. Absorbance was measured at 390 nm using a microplate reader. H_2O_2 content was expressed as $\mu\text{mol g}^{-1}$ seed weight.

Nitric oxide (NO) production was estimated indirectly using the Griess reagent method following Hasanuzzaman et al. [40] with minor modifications. Briefly, 0.2 g of sample was homogenized in 50 mM acetate buffer (pH 3.6) and centrifuged at $10,000 \times g$ for 15 min. The supernatant was mixed with Griess reagent and incubated at room temperature for 10 min. Absorbance was measured at 540 nm using a microplate reader. NO content was estimated from nitrite (NO_2^-) concentration using a sodium nitrite standard curve and expressed as $\mu\text{mol g}^{-1}$ seed weight.

Malondialdehyde (MDA) content was determined using the thiobarbituric acid (TBA) method according to da Silva et al. [41]. A 0.2 g sample was extracted in 0.1% (*w/v*) trichloroacetic acid (TCA) and reacted with TBA reagent at 95 °C for 30 min. After centrifugation, absorbance was measured at 532 and 600 nm using a microplate reader. MDA

concentration was calculated from the difference between A_{532} and A_{600} using an extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$ and expressed as nmol g^{-1} seed weight.

Proline content was determined according to Bates et al. [42] with minor modifications. A 0.1 g sample was extracted in 3% (*w/v*) sulfosalicylic acid and centrifuged at $12,000\times g$ for 10 min. The supernatant was reacted with acid ninhydrin and glacial acetic acid, incubated at 100°C for 1 h, and rapidly cooled. The chromophore was extracted with toluene, and absorbance was measured at 520 nm using a microplate reader. Proline concentration was determined using an L-proline standard curve and expressed as $\mu\text{mol g}^{-1}$ seed weight.

2.7. Antioxidant Enzyme Assays

Antioxidant enzymes were extracted under ice-cold conditions from dried seed powder. For catalase (CAT) and ascorbate peroxidase (APX) assays, 0.2 g of sample was homogenized in 50 mM sodium phosphate buffer (pH 7.0) containing polyvinylpyrrolidone (PVP) and centrifuged at $15,000\times g$ for 15 min at 4°C . The resulting supernatant was used as the crude enzyme extract.

CAT activity was determined according to Önder et al. [43] by monitoring H_2O_2 decomposition at 240 nm and expressed as U mg^{-1} protein. APX activity was measured following Heshmati et al. [44] based on the decrease in absorbance at 290 nm due to ascorbate oxidation and expressed as U mg^{-1} protein.

For superoxide dismutase (SOD) analysis, 0.5 g of sample was extracted in 100 mM sodium phosphate buffer (pH 7.8) containing PVP and centrifuged under the same conditions [40]. SOD activity was determined according to Zhang et al. [45] based on inhibition of nitroblue tetrazolium (NBT) photoreduction at 560 nm and expressed as U mg^{-1} protein.

Protein content was determined using the Bradford method, and enzyme activities were normalized accordingly.

2.8. Metabolic Analysis

α -Amylase activity was determined using a commercial assay kit (K-CERA; Megazyme, Bray, Ireland) according to the method of McCleary et al. [46]. A 3 g sample was extracted in 0.1 M sodium maleate buffer (pH 5.4) at 40°C for 20 min and centrifuged at $12,000\times g$ for 20 min. The supernatant was reacted with amylase HR reagent, and absorbance was measured at 400 nm using a microplate reader. Enzyme activity was calculated according to the manufacturer's instructions and expressed as Ceralpha Units (CU) g^{-1} seed weight.

Total soluble sugars (TSS) were determined using a modified phenol–sulfuric acid method [47]. A 0.05 g sample was extracted with 80% ethanol at 30°C for 24 h and centrifuged at $12,000\times g$ for 20 min. The supernatant was reacted with 5% phenol and concentrated sulfuric acid, and the mixture was allowed to stand for 20 min. Absorbance was measured at 490 nm using a microplate reader. TSS content was expressed as mg glucose equivalents (GE) g^{-1} seed weight.

2.9. Statistical Analysis

Analysis of variance (ANOVA) was performed to evaluate the effects of PAW priming treatments on all measured parameters. When significant differences were detected ($p < 0.05$), means were compared using Tukey's honestly significant difference (HSD) test. All analyses were conducted using IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA).

Principal component analysis (PCA) was performed using R software (version 4.5.3; R Core Team, Vienna, Austria) to evaluate multivariate relationships among PAW physi-

cochemical properties, biochemical responses, and germination and seedling traits. Prior to analysis, all variables were standardized to zero mean and unit variance. PCA was conducted using the `prcomp()` function, and the first two principal components were used for interpretation based on their contribution to total variance. Confidence ellipses (95%) were generated on PCA score plots to visualize clustering among treatments.

3. Results

3.1. Physicochemical Properties of PAW

The physicochemical properties of PAW were significantly influenced by activation time (Table 1). EC increased progressively with increasing activation time, reaching the highest value in PAW120, followed by PAW60 and PAW30, while PAW15 exhibited the lowest EC. No EC was detected in DIW under the present measurement conditions, indicating negligible ionic accumulation compared with PAW treatments. In contrast, pH decreased markedly with increasing activation time, with PAW120 showing the lowest value. DIW and PAW15 maintained near-neutral pH and were not significantly different, suggesting that short activation times had limited effects on acidification. The concentrations of H_2O_2 and NO_3^- increased consistently with activation time, indicating enhanced accumulation of RONS under prolonged plasma exposure. PAW120 showed the highest levels of both species, whereas only trace amounts were detected in PAW15, and none were detected in DIW. In contrast, NO_2^- exhibited a different pattern, reaching a maximum at PAW30 and decreasing at longer activation times (PAW60 and PAW120). Thus, PAW30 was characterized by an intermediate RONS profile with the highest NO_2^- concentration, whereas PAW60 and PAW120 showed a shift toward higher H_2O_2 and NO_3^- accumulation.

Table 1. Physicochemical properties and RONS of PAW generated at different activation times.

Treatment ¹	EC ² ($\mu\text{S cm}^{-1}$)	pH	H_2O_2 (mg L^{-1})	NO_2^- (mg L^{-1})	NO_3^- (mg L^{-1})
DIW	0.00 $\pm$ 0.00 ^{e3}	7.11 $\pm$ 0.07 ^a	0.00 $\pm$ 0.00 ^e	0.00 $\pm$ 0.00 ^c	0.00 $\pm$ 0.00 ^e
PAW15	84.50 $\pm$ 2.87 ^d	7.05 $\pm$ 0.05 ^a	0.22 $\pm$ 0.04 ^d	0.14 $\pm$ 0.00 ^b	2.68 $\pm$ 0.02 ^d
PAW30	102.50 $\pm$ 2.29 ^c	3.88 $\pm$ 0.02 ^b	5.80 $\pm$ 0.06 ^c	5.25 $\pm$ 0.04 ^a	5.01 $\pm$ 0.06 ^c
PAW60	122.25 $\pm$ 1.78 ^b	3.25 $\pm$ 0.04 ^c	9.11 $\pm$ 0.08 ^b	0.05 $\pm$ 0.02 ^c	8.12 $\pm$ 0.04 ^b
PAW120	133.25 $\pm$ 3.34 ^a	3.13 $\pm$ 0.01 ^d	12.49 $\pm$ 0.07 ^a	0.03 $\pm$ 0.00 ^c	8.76 $\pm$ 0.01 ^a
C.V.%	3.08	0.92	3.61	2.89	0.91

¹ DIW, distilled water; PAW15–PAW120, PAW with activation times of 15–120 s. ² EC, electrical conductivity; H_2O_2 , hydrogen peroxide; NO_2^- , nitrite; NO_3^- , nitrate. ³ Values are means $\pm$ SD ($n = 4$). Different letters within the same column indicate significant differences according to Tukey's HSD test ($p < 0.05$).

3.2. Biochemical Responses of Hot Pepper Seeds to PAW Priming

PAW priming significantly affected the biochemical responses of hot pepper seeds (Table 2). H_2O_2 content increased with increasing PAW activation time, reaching the highest level in PAW120, followed by PAW60, whereas PAW30, PAW15, and DIW exhibited similarly low levels. NP showed the lowest H_2O_2 content. NO exhibited a distinct response pattern, with the highest level observed in PAW60, while PAW120 and PAW30 showed comparable but lower levels, and DIW and PAW15 displayed relatively low NO content. The lowest level was observed in NP. A similar trend to that of H_2O_2 was observed for both MDA and proline, with the highest levels detected in PAW120, followed by PAW60, whereas DIW, PAW15, and PAW30 exhibited comparably low levels, and NP showed the lowest contents. MDA and proline were markedly elevated in PAW120, indicating that prolonged activation imposed a greater biochemical stress response. PAW30, however,

maintained both parameters at levels similar to DIW and PAW15, despite showing the best germination performance.

Table 2. Effects of PAW priming on biochemical responses in hot pepper seeds.

Treatment ¹	H ₂ O ₂ ² ($\mu\text{mol g}^{-1}$ Seed Weight)	NO ($\mu\text{mol g}^{-1}$ Seed Weight)	MDA (nmol g^{-1} Seed Weight)	Proline ($\mu\text{mol g}^{-1}$ Seed Weight)
NP	0.61 $\pm$ 0.00 ^{e3}	18.60 $\pm$ 0.84 ^e	0.08 $\pm$ 0.03 ^d	0.58 $\pm$ 0.04 ^d
DIW	0.86 $\pm$ 0.01 ^d	21.67 $\pm$ 0.92 ^d	0.32 $\pm$ 0.00 ^c	1.10 $\pm$ 0.03 ^c
PAW15	0.87 $\pm$ 0.01 ^{cd}	23.31 $\pm$ 0.61 ^{cd}	0.31 $\pm$ 0.03 ^c	1.10 $\pm$ 0.07 ^c
PAW30	0.93 $\pm$ 0.03 ^c	24.77 $\pm$ 0.48 ^{bc}	0.34 $\pm$ 0.03 ^c	1.10 $\pm$ 0.04 ^c
PAW60	1.27 $\pm$ 0.07 ^b	34.05 $\pm$ 0.97 ^a	0.40 $\pm$ 0.03 ^b	1.21 $\pm$ 0.01 ^b
PAW120	1.74 $\pm$ 0.02 ^a	26.15 $\pm$ 0.46 ^b	0.85 $\pm$ 0.03 ^a	1.80 $\pm$ 0.03 ^a
C.V.%	3.02	3.46	8.23	3.88

¹ NP, non-primed seeds; DIW, seeds primed with distilled water; PAW15–PAW120, seeds primed with PAW generated at activation times of 15–120 s. ² H₂O₂, hydrogen peroxide; NO, nitric oxide; MDA, malondialdehyde. ³ Values are means $\pm$ SD ($n = 4$). Different letters within the same column indicate significant differences according to Tukey's HSD test ($p < 0.05$).

3.3. Antioxidant Enzyme Activities in Response to PAW Priming

PAW priming significantly enhanced antioxidant enzyme activities in hot pepper seeds (Table 3). SOD and CAT activities were strongly stimulated by PAW30 and PAW60, with both treatments showing statistically comparable values. Lower but comparable SOD activities were observed in PAW15, DIW, and PAW120, whereas NP showed the lowest activity. For CAT, PAW120 maintained higher activity than DIW and PAW15 but remained lower than PAW30 and PAW60. In contrast, APX activity peaked in PAW30, with no significant differences compared with PAW15 and PAW60. Lower APX activity was observed in PAW120 and DIW, whereas NP showed the lowest level. The antioxidant enzyme profile was therefore most pronounced under PAW30 and PAW60, whereas PAW120 showed a partial decline, particularly for SOD and APX.

Table 3. Effects of PAW priming on antioxidant enzyme activities in hot pepper seeds.

Treatment ¹	SOD ² (U mg^{-1} Protein)	CAT (U mg^{-1} Protein)	APX (U mg^{-1} Protein)
NP	13.51 $\pm$ 1.21 ^{c3}	2.37 $\pm$ 0.13 ^d	11.90 $\pm$ 1.29 ^d
DIW	19.95 $\pm$ 0.57 ^b	4.21 $\pm$ 0.52 ^c	16.10 $\pm$ 1.08 ^c
PAW15	20.05 $\pm$ 0.84 ^b	4.33 $\pm$ 0.32 ^c	19.32 $\pm$ 0.97 ^{ab}
PAW30	23.06 $\pm$ 0.81 ^a	6.28 $\pm$ 0.24 ^a	21.62 $\pm$ 1.57 ^a
PAW60	24.11 $\pm$ 1.21 ^a	6.38 $\pm$ 0.15 ^a	20.19 $\pm$ 0.30 ^{ab}
PAW120	19.11 $\pm$ 0.79 ^b	5.65 $\pm$ 0.07 ^b	18.15 $\pm$ 0.77 ^{bc}
C.V.%	5.40	6.68	6.93

¹ NP, non-primed seeds; DIW, seeds primed with distilled water; PAW15–PAW120, seeds primed with PAW generated at activation times of 15–120 s. ² SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase. ³ Values are means $\pm$ SD ($n = 4$). Different letters within the same column indicate significant differences according to Tukey's HSD test ($p < 0.05$).

3.4. PAW Priming Enhances Metabolic Activation During Germination

PAW priming enhanced metabolic activity in hot pepper seeds, as indicated by increased α -amylase activity and TSS content (Table 4). α -amylase activity was highest in PAW30, followed by PAW60, while PAW15 and DIW showed moderate levels. In contrast, PAW120 exhibited relatively low activity, and NP showed the lowest α -amylase activity. A similar trend was observed for TSS, where PAW30 showed the highest levels,

comparable to PAW60. PAW120 exhibited moderate sugar accumulation, whereas PAW15 and DIW showed lower levels. The lowest sugar content was observed in NP. The strongest metabolic response was observed in PAW30, as shown by the highest α -amylase activity and TSS content. PAW120, by contrast, showed lower α -amylase activity despite maintaining moderate soluble sugar accumulation.

Table 4. Effects of PAW priming on metabolic activity in hot pepper seeds.

Treatment ¹	α -Amylase (CU g ⁻¹ Seed Weight)	TSS (mg GE g ⁻¹ Seed Weight)
NP	0.19 ± 0.01 ^{f2}	2.24 ± 0.04 ^d
DIW	0.27 ± 0.01 ^d	2.43 ± 0.02 ^c
PAW15	0.31 ± 0.01 ^c	2.42 ± 0.08 ^c
PAW30	0.40 ± 0.00 ^a	3.50 ± 0.09 ^a
PAW60	0.35 ± 0.01 ^b	3.34 ± 0.09 ^a
PAW120	0.25 ± 0.02 ^e	2.90 ± 0.04 ^b
C.V.%	2.62	2.76

¹ NP, non-primed seeds; DIW, seeds primed with distilled water; PAW15–PAW120, seeds primed with PAW generated at activation times of 15–120 s. ² Values are means ± SD ($n = 4$). Different letters within the same column indicate significant differences according to Tukey's HSD test ($p < 0.05$).

3.5. Effects of PAW Priming on Germination Performance, Seed Vigor, and Seedling Growth

PAW priming significantly improved germination performance, seed vigor, and early seedling growth in hot pepper compared with NP and DIW controls (Tables 5 and 6). GP were markedly enhanced by PAW priming, with PAW30 showing the highest value. In contrast, PAW15, PAW60, PAW120, and DIW showed moderate responses, while NP exhibited the lowest performance (Table 5). Germination dynamics were significantly influenced by PAW priming. PAW30 resulted in the fastest germination, as indicated by reduced MGT. In addition, PAW30 showed the highest GI and improved GAA. PAW15 and PAW60 exhibited intermediate responses, while PAW120 and DIW showed reduced improvements. Although PAW60 and PAW120 maintained GP above NP, their lower GI and GAA values relative to PAW30 indicate that prolonged activation did not provide the same vigor advantage as the 30 s treatment.

Table 5. Effects of PAW priming on germination and vigor in hot pepper seeds.

Treatment ¹	GP ² (%)	MGT (Days)	GI	GAA (%)
NP	86.50 ± 1.66 ^{c3}	10.58 ± 0.05 ^a	4.04 ± 0.05 ^d	76.50 ± 1.65 ^c
DIW	89.00 ± 1.00 ^{bc}	9.68 ± 0.05 ^b	4.82 ± 0.06 ^c	80.00 ± 1.41 ^b
PAW15	90.00 ± 1.41 ^b	9.35 ± 0.15 ^c	5.13 ± 0.06 ^b	80.00 ± 0.00 ^b
PAW30	95.00 ± 1.00 ^a	8.98 ± 0.10 ^d	5.48 ± 0.11 ^a	84.50 ± 0.87 ^a
PAW60	90.50 ± 1.66 ^b	9.57 ± 0.12 ^{bc}	4.78 ± 0.12 ^c	78.00 ± 1.41 ^{bc}
PAW120	90.50 ± 0.87 ^b	9.68 ± 0.03 ^b	4.79 ± 0.16 ^c	77.00 ± 1.00 ^{bc}
C.V.%	1.67	1.14	2.45	1.73

¹ NP, non-primed seeds; DIW, seeds primed with distilled water; PAW15–PAW120, seeds primed with PAW generated at activation times of 15–120 s. ² GP, germination percentage; MGT, mean germination time; GI, germination index; GAA, germination percentage after accelerated aging. ³ Values are means ± SD ($n = 4$). Different letters within the same column indicate significant differences according to Tukey's HSD test ($p < 0.05$).

Seedling growth parameters, including shoot length, fresh weight, and dry weight, were generally enhanced by PAW priming, with PAW30 showing the most pronounced effects (Table 6). Root length did not show significant differences among treatments.

Table 6. Effects of PAW priming on seedling growth in hot pepper.

Treatment ¹	Shoot Length (mm)	Root Length (mm)	Fresh Weight (mg)	Dry Weight (mg)
NP	23.79 ± 0.68 ^{b2}	32.92 ± 3.93 ^a	21.37 ± 0.72 ^c	1.38 ± 0.03 ^d
DIW	23.93 ± 0.83 ^b	36.11 ± 2.19 ^a	21.03 ± 0.94 ^c	1.51 ± 0.06 ^c
PAW15	23.95 ± 0.51 ^b	38.48 ± 0.84 ^a	22.37 ± 0.45 ^{bc}	1.66 ± 0.06 ^b
PAW30	26.58 ± 0.96 ^a	39.68 ± 3.56 ^a	25.79 ± 0.67 ^a	1.82 ± 0.06 ^a
PAW60	23.38 ± 0.54 ^b	38.23 ± 1.74 ^a	24.11 ± 0.75 ^b	1.78 ± 0.01 ^{ab}
PAW120	21.58 ± 0.26 ^c	33.79 ± 2.58 ^a	21.69 ± 0.17 ^{bc}	1.75 ± 0.03 ^{ab}
C.V.%	3.24	8.49	3.58	3.32

¹ NP, non-primed seeds; DIW, seeds primed with distilled water; PAW15–PAW120, seeds primed with PAW generated at activation times of 15–120 s. ² Values are means ± SD ($n = 4$). Different letters within the same column indicate significant differences according to Tukey's HSD test ($p < 0.05$).

3.6. Principal Component Analysis (PCA)

PCA was conducted to evaluate the relationships among PAW properties, biochemical responses, and germination performance. For PAW properties, PC1 and PC2 explained 74.5% and 20.6% of the total variance, respectively (Figure 2). The score plot (Figure 2a) revealed distinct treatment separation along PC1, with DIW and PAW15 located on the negative side and PAW60 and PAW120 on the positive side, reflecting the progressive shift in PAW chemistry with longer activation times. PAW30 was separated along PC2 on the negative side, indicating that this treatment had a distinct chemical profile compared with both short- and long-duration activation treatments. The loading plot (Figure 2b) showed that NO_3^- , H_2O_2 , and EC were positively associated with PC1, whereas pH was negatively associated with this component. NO_2^- contributed strongly to the separation of PAW30 along PC2, consistent with its peak concentration in this treatment.

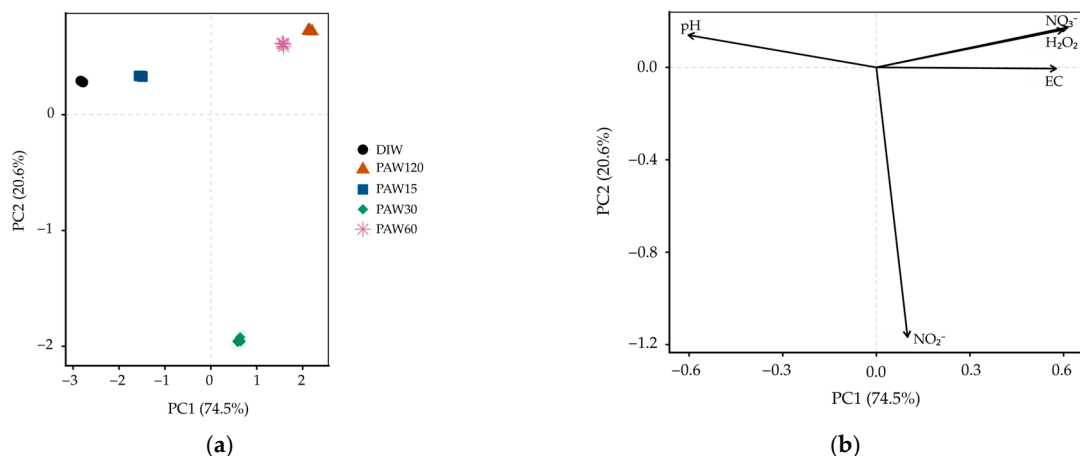


Figure 2. Principal component analysis (PCA) of plasma-activated water (PAW) physicochemical properties. (a) Score plot of treatments. (b) Loading plot of physicochemical properties and reactive oxygen and nitrogen species (RONS).

For biochemical responses, PC1 and PC2 explained 66.3% and 23.6% of the total variance, respectively (Figure 3). NP was clearly separated on the negative side of PC1. PAW120 was located in the positive PC2 region, whereas PAW30 and PAW60 were located in the negative PC2 region, despite all three treatments being located in the positive PC1 region. DIW and PAW15 were clustered near the center (Figure 3a). The loading plot (Figure 3b) indicated that MDA, H_2O_2 , and proline were positively associated with both PC1 and PC2, while antioxidant enzymes (SOD, APX, and CAT), together with TSS, were associated with positive PC1 and negative PC2. Amylase showed a negative contribution to PC2. PAW120 was positioned near MDA, H_2O_2 , and proline loadings. Conversely,

PAW30 and PAW60 were closer to the loadings for antioxidant enzymes, TSS, and α -amylase.

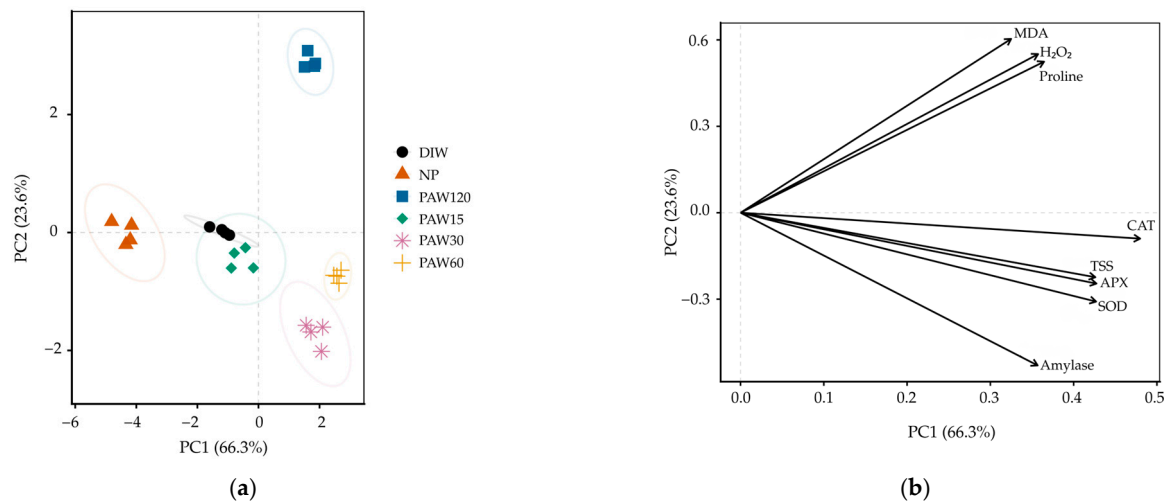


Figure 3. Principal component analysis (PCA) of biochemical responses in hot pepper seeds under PAW priming. (a) Score plot of treatments. (b) Loading plot of biochemical parameters.

For germination performance, PC1 and PC2 explained 65.2% and 14.7% of the total variance, respectively (Figure 4). The score plot (Figure 4a) showed a clear separation along PC1, with NP on the negative side and PAW30 on the positive side. The other treatments were distributed near the center, with partial separation along PC2. The loading plot (Figure 4b) indicated that GI, GP, and FW were positively associated with PC1, while MGT was negatively associated. RL and GAA were associated with positive PC1 and negative PC2, whereas DW was associated with the positive directions of both PC1 and PC2. SL showed a negative contribution to PC2. PAW30 was closely associated with GP, GI, FW, and GAA. In contrast, NP was associated with MGT, indicating delayed and less vigorous germination.

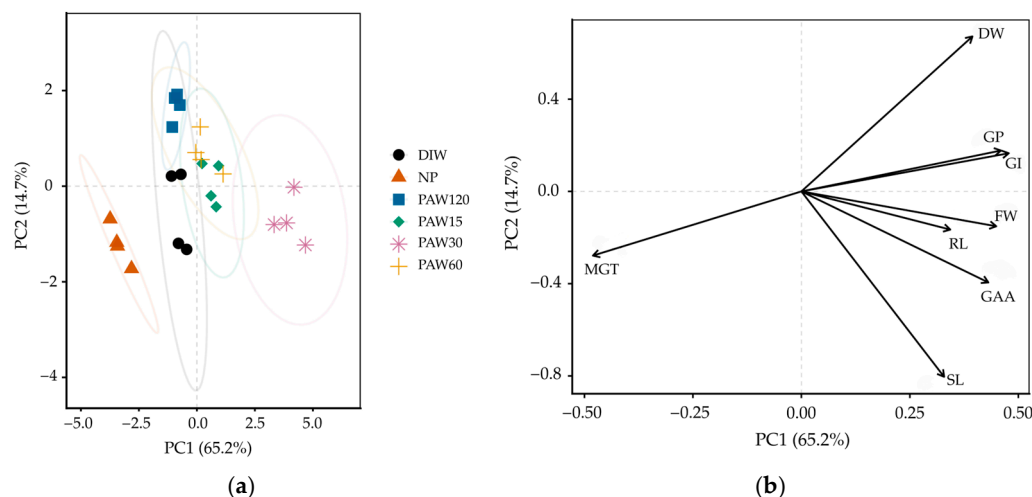


Figure 4. Principal component analysis (PCA) of germination performance in hot pepper seeds under PAW priming. (a) Score plot of treatments. (b) Loading plot of germination and seedling traits.

4. Discussion

The observed changes in PAW physicochemical properties are primarily governed by plasma–liquid interactions, which drive the formation of RONS through complex gas–liquid phase reactions and interfacial mass transfer processes [48]. These processes lead to

the accumulation of long-lived species such as H_2O_2 , NO_2^- , and NO_3^- in the liquid phase, depending on plasma conditions and solution chemistry [11,49]. Furthermore, the interaction pathways and resulting chemical composition can be significantly influenced by the presence of organic matter and interfacial reaction dynamics [50]. In the present study, longer activation times progressively increased EC, H_2O_2 , and NO_3^- , while lowering pH, indicating greater accumulation of ionic and acidic reaction products. This trend is consistent with previous studies on time-dependent plasma activation [22,23]. In contrast, NO_2^- concentration peaked at intermediate activation time (30 s) and declined with prolonged treatment (60 and 120 s), likely due to its conversion into the more stable NO_3^- via acid-driven aqueous-phase reaction pathways involving H_2O_2 and reactive nitrogen intermediates [10,51], as similarly reported by Lee et al. [52]. The decrease in pH with longer activation time was likely related to the formation of nitric acid and other acidic intermediates derived from dissolved nitrogen oxides [48,53,54].

When applied as a priming agent, PAW likely altered the redox environment surrounding imbibed hot pepper seeds, as reflected by changes in seed H_2O_2 and NO contents. H_2O_2 can diffuse across cell membranes through aquaporins, particularly peroxiporins, enabling its intracellular accumulation and signaling function [55–57]. In parallel, NO_3^- is transported through specific nitrate transporters, contributing to downstream NO biosynthesis within seeds [58–60]. At optimal concentrations, H_2O_2 and NO function as key signaling molecules that regulate seed germination through redox-mediated crosstalk with phytohormones, particularly gibberellins (GA) and abscisic acid (ABA), thereby playing a crucial role in breaking seed dormancy and promoting germination [61–63]. In this study, improved germination under PAW30 was associated with moderate H_2O_2 and NO levels, enhanced antioxidant enzyme and α -amylase activities, and increased soluble sugar accumulation, indicating coordinated metabolic activation during early germination.

The present findings support a dose-dependent hormetic effect of PAW priming, in which moderate plasma activation (PAW30) stimulated beneficial physiological and biochemical responses, whereas prolonged activation durations (PAW60 and PAW120) reduced the magnitude of improvement and shifted seed responses toward a less favorable redox status [20]. This response pattern is consistent with the hormesis model, in which moderate levels of ROS/RNS act as signaling molecules that activate defense and metabolic pathways, whereas excessive accumulation disrupts cellular homeostasis and impairs seed metabolism [16,17]. The biochemical profile observed under PAW30 further supports this hormetic interpretation, as evidenced by moderate H_2O_2 and NO levels, together with low MDA and proline accumulation (Table 2), suggesting that redox changes remained within a range compatible with seed metabolic activation. Enhanced activities of antioxidant enzymes (SOD, CAT, and APX; Table 3), together with limited lipid peroxidation and low proline accumulation, further indicate a balanced antioxidant response under PAW30. Such a response may have helped maintain cellular stability and supported germination performance and seedling establishment (Tables 5 and 6) [16,17]. Although all PAW solutions exhibited lower pH values than DIW, the superior performance of the PAW30 treatment compared with the PAW120 treatment suggests that germination responses were not governed solely by solution acidity. Rather, the results support a redox-mediated hormetic response associated with plasma-generated RONS, which are known to function as important signaling molecules during seed germination and early seedling development [6,17,18]. Further studies using pH-adjusted controls would help distinguish the effects of acidity from those of PAW-derived reactive species. These findings are consistent with previous reports showing that moderate ROS levels enhance antioxidant defense systems and improve seed performance [64,65], and align with PAW-based priming studies demonstrating improved growth under controlled oxidative conditions [31,66]. Similar stimulatory effects of PAW on seed germination and early seedling

development have also been reported in mung bean and lettuce, where PAW treatment enhanced germination rate, seedling vigor, and plant growth in association with improved physiological activity [29,30]. Consistent with these findings, diluted PAW has also been shown to promote germination and early growth in various sprout crops, further supporting the broad applicability of PAW-based seed treatments across different plant species [28]. Although the improvements in GP and MGT relative to the DIW treatment were modest, the consistent enhancement across vigor-related traits, reserve mobilization, antioxidant activity, and redox-associated biochemical responses suggests that the benefits of PAW extend beyond germination percentage alone and involve broader physiological regulation during early seedling establishment.

Increasing PAW activation time (PAW60 and PAW120) resulted in a marked rise in H_2O_2 and NO levels (Table 2), accompanied by elevated MDA and proline contents, indicating a stronger redox- and stress-related biochemical response. The pronounced accumulation of these stress markers in PAW120, together with higher NO_3^- concentrations and lower pH (Table 1), suggests that prolonged activation shifted the primed seeds toward a less favorable oxidative status, as reflected by higher lipid peroxidation [67,68]. Although SOD and CAT activities were high under PAW60, their antioxidant capacity did not coincide with the best germination performance, indicating that a strong antioxidant response alone was not sufficient to produce the most favorable seed vigor [16,17]. At prolonged activation (PAW120), the decline in antioxidant enzyme activities may indicate oxidative impairment of cellular components, potentially involving protein carbonylation, enzyme inactivation, and disruption of redox-sensitive metabolic processes [69–71]. Similar less favorable responses under prolonged PAW exposure have also been reported in previous PAW studies, where prolonged plasma treatment negatively affected germination and seedling development in association with excessive reactive species accumulation [29,30]. Overall, these findings support the hypothesis that PAW priming induces treatment-dependent redox responses, with a shift from a favorable redox window under PAW30 toward a less optimal biochemical state at longer activation times.

Metabolic responses further supported the dose-dependent effects of PAW priming. Among all treatments, PAW30 induced the strongest metabolic response, as reflected by increased α -amylase activity and TSS accumulation, followed by PAW60, whereas PAW15 and DIW showed comparatively moderate responses and PAW120 exhibited lower α -amylase activity than the other PAW treatments (Table 4). This pattern suggests that PAW30 provided favorable conditions for reserve mobilization, possibly through a balanced redox environment in primed seeds. Consistent with recent findings in tef, gas PAW priming has been shown to enhance seed metabolic activity and germination in association with redox-related responses [31]. Moderate RONS levels, particularly NO-related responses, have been linked to enzyme activation and cellular protection, which may help explain the higher α -amylase activity and promoting starch hydrolysis into soluble sugars [72]. Enhanced carbohydrate mobilization under PAW30 may therefore contribute to the improved germination rate and seedling vigor observed in this study, since efficient starch degradation is essential for energy production during early germination [73]. Conversely, the reduced α -amylase activity and soluble sugar accumulation observed under PAW120 and, to a lesser extent, PAW60 suggest that excessive RONS negatively affected metabolic function. This response may be associated with a less favorable oxidative status, as indicated by higher H_2O_2 , MDA, and proline levels. Consequently, the lower α -amylase activity under prolonged activation may partly explain the weaker germination vigor compared with PAW30. Similar less favorable responses under prolonged PAW exposure have been reported in previous PAW studies, where prolonged plasma exposure disrupted metabolic homeostasis and suppressed seedling development [29,30].

The relatively weak physiological activation observed under PAW15 may reflect insufficient RONS accumulation, as indicated by the low concentrations of H_2O_2 , NO_2^- , and NO_3^- together with the near-neutral pH (Table 1). These conditions may not have been sufficient to effectively activate the metabolic responses associated with improved germination performance. Similar responses have been reported in PAW-treated seedlings, where suboptimal reactive species levels resulted in limited metabolic stimulation and reduced physiological activation [32].

The physiological and metabolic responses observed in this study reinforce the concept of a hormetic response, in which an optimal range of RONS enhances metabolic activation and seed performance, whereas both insufficient and excessive reactive species levels result in suboptimal physiological outcomes [16,17,20]. This biphasic response emphasizes the importance of optimizing PAW generation conditions to achieve a balance between redox-mediated stimulation and stress-related inhibition [17,20]. Although the present study demonstrates clear treatment-dependent responses, the specific contribution of individual ROS and RNS remains difficult to distinguish because these reactive compounds may interact dynamically during PAW priming. To further elucidate the relationships among physicochemical properties, biochemical responses, and germination performance, principal component analysis (PCA) was conducted.

The PCA models (Figures 2–4) supported associations among PAW physicochemical properties, redox-associated biochemical responses, and germination performance in hot pepper seeds. The distribution of PAW treatments along PC1, with PAW60 and PAW120 positioned toward the positive region, appeared to reflect the degree of plasma activation, which was associated with increasing NO_3^- , H_2O_2 , and EC, together with decreasing pH (Figure 2). These variables were consistent with progressive RONS accumulation with prolonged activation time. The strong contribution of these parameters to PC1 suggests that plasma-induced chemical modifications of water were closely associated with treatment separation in the PCA model. Since RONS play central roles in redox signaling and stress regulation, the observed separation pattern may reflect differences in the redox-related biochemical status of primed seeds [17,40].

This physicochemical gradient was accompanied by changes in seed biochemical responses, as reflected by the grouping of PAW-primed seeds along the positive region of PC1 (Figure 3). The positive loading of MDA, H_2O_2 , and proline on PC1 and PC2 indicates that prolonged plasma activation, particularly PAW120 located in the positive PC2 region, was associated with higher levels of stress-related biochemical markers. In contrast, PAW30 and PAW60, which were positioned in the positive PC1 and negative PC2 region, were located nearer to the loadings for antioxidant enzymes (SOD, CAT, and APX), together with TSS and α -amylase activity, suggesting stronger antioxidant and metabolic responses under these treatments. The simultaneous association of oxidative stress markers and antioxidant activities suggests that PAW priming induced a treatment-dependent redox response rather than a uniform increase in oxidative stress [16,17]. In particular, the association of antioxidant enzymes and α -amylase activity with favorable germination traits suggests that balanced redox signaling may promote reserve mobilization and germination metabolism [6,17].

These biochemical adjustments were consistent with the germination and seedling responses shown in Figure 4. The positioning of PAW30 with GI, GP, and FW on the positive side of PC1, together with the negative association of MGT, indicates that moderate levels of reactive species were associated with enhanced germination efficiency and seedling vigor, consistent with previous findings [6]. In contrast, the distinct separation of PAW120 along PC2 suggests that excessive RONS accumulation was linked with a less favorable germination profile than PAW30. This pattern supports the hormetic nature of PAW responses, in which seed performance improves within an optimal redox range but

declines when activation becomes excessive [17,20,25]. The dual role of ROS/RNS as both signaling molecules and stress inducers has been widely recognized in plant redox biology [17,40]. Interestingly, PAW120 reduced shoot length, root length, and fresh weight compared with PAW30, whereas dry weight remained statistically comparable to PAW30 and PAW60 and significantly higher than the control treatments. This finding suggests that excessive plasma activation may have inhibited seedling elongation to a greater extent than biomass accumulation. Such a response is consistent with hormetic stress effects, whereby elevated levels of reactive species suppress growth-related processes while inducing physiological and metabolic adjustments through redox-mediated regulation of plant development [19–21]. Moreover, although the difference in dry weight between the DIW and PAW30 treatments was relatively small, PAW30 consistently improved multiple physiological and biochemical parameters, including germination performance, seedling vigor, reserve mobilization, and redox-related responses, indicating that its biological effects were not limited to biomass accumulation alone. However, because seedling moisture content and biomass composition were not determined, the physiological basis underlying this response remains unclear and warrants further investigation.

Collectively, these results support a hormetic response to PAW priming, in which an optimal redox window enhances seed performance, whereas both insufficient and excessive reactive species accumulation result in suboptimal physiological outcomes. To further integrate these findings, a conceptual model summarizing the relationships among PAW properties, seed biochemical responses, and germination performance is presented in Figure 5. Low plasma activation (PAW15) generated relatively low levels of RONS, resulting in limited redox signaling and weaker stimulation of germination-related metabolic processes. Moderate plasma activation (PAW30) generated optimal levels of RONS, which promoted antioxidant regulation and reserve mobilization, leading to enhanced germination and seed vigor. In contrast, excessive plasma activation (PAW120) induced oxidative stress, as indicated by elevated H_2O_2 , MDA, and proline levels, thereby reducing germination performance. This model provides a conceptual framework for understanding the dose-dependent effects of PAW priming in hot pepper seeds.

However, the present study was limited to physiological and biochemical assessments and did not directly examine phytohormonal regulation during PAW-induced germination. Seed germination is governed by complex interactions among RONS, antioxidant systems, and phytohormones, particularly GA and ABA. Future studies should therefore investigate changes in GA and ABA levels and their interactions with RONS, together with related gene expression and antioxidant regulation, to clarify the mechanisms underlying PAW-mediated hormetic responses in hot pepper seeds. In addition, TSS content was used as a general indicator of carbohydrate mobilization during germination; however, the contributions of individual sugars were not determined. Targeted carbohydrate profiling, particularly of raffinose family oligosaccharides together with sucrose, glucose, and fructose, would provide a more detailed understanding of carbohydrate metabolism and its role in PAW-induced seed vigor and germination responses. Despite these limitations, the present findings provide important insights into the physiological basis of PAW priming and support its potential application as a sustainable strategy for improving seed vigor and germination performance.

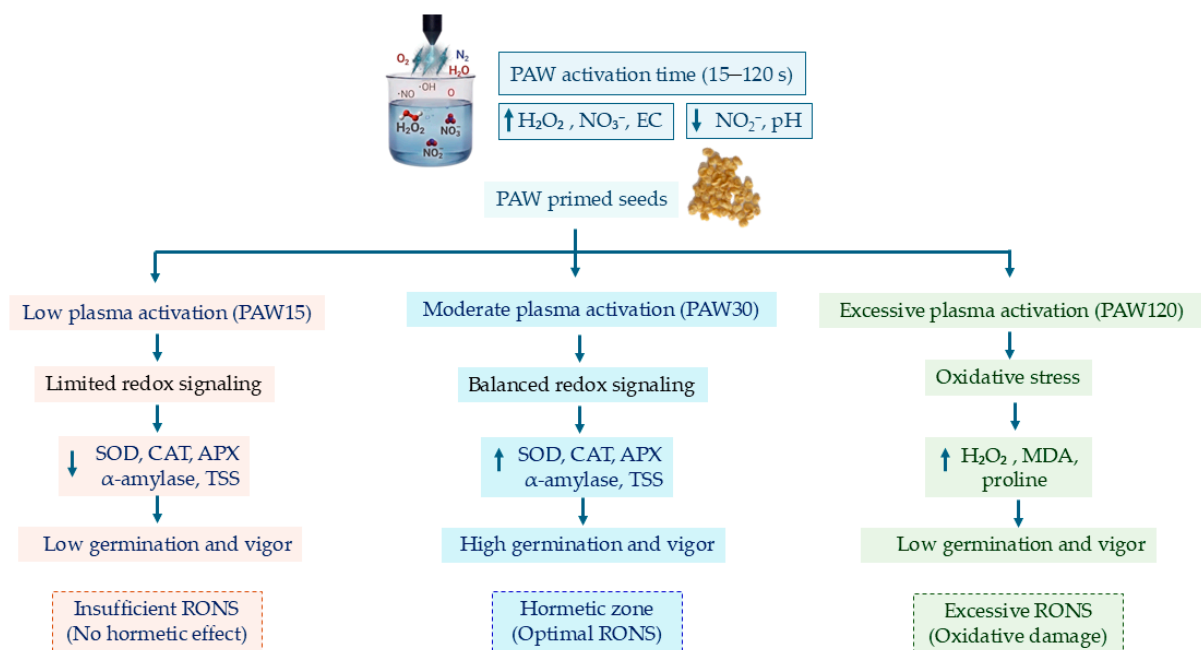


Figure 5. Conceptual model illustrating PAW-induced hormetic regulation in hot pepper seeds, integrating PAW activation levels, RONS generation, biochemical responses, and germination performance. Arrows between boxes indicate the proposed sequence of events, while upward (↑) and downward (↓) arrows indicate increased and decreased responses, respectively.

5. Conclusions

This study demonstrates that PAW priming effects in hot pepper seeds are strongly associated with plasma activation-induced changes in physicochemical properties, which subsequently influence the redox-related biochemical status of primed seeds. Low plasma activation (PAW15) generated relatively low levels of reactive species and provided limited stimulation of seed metabolic responses. In contrast, moderate plasma activation (PAW30) established a favorable redox environment that enhanced germination performance and seedling vigor. Prolonged plasma activation (PAW60 and PAW120), however, resulted in excessive reactive species accumulation, elevated oxidative stress-related biochemical indicators, and less favorable seed vigor responses relative to PAW30. These findings demonstrate a dose-dependent hormetic response underlying the effects of PAW priming. The integrated analysis of PAW physicochemical properties, biochemical responses, and germination traits highlights the importance of maintaining an optimal redox balance for seed metabolic activation and successful germination. Furthermore, the conceptual model proposed in this study provides a framework for understanding the relationships among PAW properties, redox regulation, reserve mobilization, and germination performance. Overall, PAW priming represents a promising eco-friendly strategy for improving seed establishment in hot pepper and potentially other horticultural crops.

Author Contributions: Conceptualization, P.R.; methodology, P.R., Y.J., B.H. and N.P.; software, P.R.; validation, P.R., C.N., Y.J. and N.P.; formal analysis, P.R. and C.N.; investigation, P.R., C.N., Y.J. and N.P.; resources, P.R., B.H. and N.P.; data curation, P.R. and C.N.; writing—original draft preparation, P.R. and C.N.; writing—review and editing, P.R., Y.J., B.H. and N.P.; visualization, P.R. and C.N.; supervision, P.R. and Y.J.; project administration, P.R.; funding acquisition, P.R. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Thammasat University Research Fund (Contract No. TUFT 005/2569).

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors sincerely acknowledge the Department of Agricultural Technology, Faculty of Science and Technology, Thammasat University, Thailand, for providing the research facilities for this study, and gratefully acknowledge the financial support from the Scholarship for Talent Students to Study in the Graduate Program, Faculty of Science and Technology, Thammasat University (Contract No. TB 16/2567), which covered the tuition fees of the student.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

APX	Ascorbate peroxidase
CAT	Catalase
DIW	Distilled water
DW	Dry weight
EC	Electrical conductivity
FW	Fresh weight
GAA	Germination percentage after accelerated aging test
GI	Germination index
GP	Germination percentage
H ₂ O ₂	Hydrogen peroxide
ISTA	International Seed Testing Association
MDA	Malondialdehyde
MGT	Mean germination time
NO	Nitric oxide
NO ₂ ⁻	Nitrite
NO ₃ ⁻	Nitrate
NP	Non-primed seeds
PAW	Plasma-activated water
PCA	Principal component analysis
RNS	Reactive nitrogen species
RONs	Reactive oxygen and nitrogen species
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TSS	Total soluble sugars
Tukey's HSD	Tukey's honestly significant difference

References

1. Pandey, S.K.; Yadav, S.K.; Singh, V.K. An overview on *Capsicum annuum* L. *JPST* **2012**, *4*, 821–828.
2. Basith, S.; Cui, M.; Hong, S.; Choi, S. Harnessing the therapeutic potential of capsaicin and its analogues in pain and other diseases. *Molecules* **2016**, *21*, 966. <https://doi.org/10.3390/molecules21080966>.
3. Palma, J.M.; Terán, F.; Contreras-Ruiz, A.; Rodríguez-Ruiz, M.; Corpas, F.J. Antioxidant profile of pepper (*Capsicum annuum* L.) fruits containing diverse levels of capsaicinoids. *Antioxidants* **2020**, *9*, 878. <https://doi.org/10.3390/antiox9090878>.
4. Silva, P.P.; Freitas, R.A.; Nascimento, W.M. Hot pepper seed priming and germination at different temperatures. *Acta Hort.* **2012**, *932*, 341–343. <https://doi.org/10.17660/ActaHortic.2012.932.49>.
5. Samarah, N.; Wang, H.; Welbaum, G.E. Pepper (*Capsicum annuum*) seed germination and vigour following priming treatments. *Seed Sci. Technol.* **2016**, *44*, 609–623. <https://doi.org/10.15258/sst.2016.44.3.18>.
6. Wojtyła, Ł.; Lechowska, K.; Kubala, S.; Garnczarska, M. Different modes of hydrogen peroxide action during seed germination. *Front. Plant Sci.* **2016**, *7*, 66. <https://doi.org/10.3389/fpls.2016.00066>.
7. Jatana, B.S.; Grover, S.; Ram, H.; Baath, G.S. Seed priming: Molecular and physiological mechanisms underlying biotic and abiotic stress tolerance. *Agronomy* **2024**, *14*, 2901. <https://doi.org/10.3390/agronomy14122901>.

8. Gao, Y.; Francis, K.; Zhang, X. Review on formation of cold plasma activated water (PAW) and the applications in food and agriculture. *Food Res. Int.* **2022**, *157*, 111246. <https://doi.org/10.1016/j.foodres.2022.111246>.
9. Sawangrat, C.; Phimolsiripol, Y.; Leksakul, K.; Thanapornpoonpong, S.-N.; Sojithamporn, P.; Lavilla, M.; Castagnini, J.M.; Barba, F.J.; Boonyawan, D. Application of pinhole plasma jet activated water against *Escherichia coli*, *Colletotrichum gloeosporioides*, and decontamination of pesticide residues on chilli (*Capsicum annuum* L.). *Foods* **2022**, *11*, 2859. <https://doi.org/10.3390/foods11182859>.
10. Thirumdas, R.; Kothakota, A.; Annapure, U.; Siliveru, K.; Blundell, R.; Gatt, R.; Valdramidis, V.P. Plasma activated water (PAW): Chemistry, physico-chemical properties, applications in food and agriculture. *Trends Food Sci. Technol.* **2018**, *77*, 21–31. <https://doi.org/10.1016/j.tifs.2018.05.007>.
11. Wong, K.S.; Chew, N.S.L.; Low, M.; Tan, M.K. Plasma-activated water: Physicochemical properties, generation techniques, and applications. *Processes* **2023**, *11*, 2213. <https://doi.org/10.3390/pr11072213>.
12. Sachdev, S.; Ansari, S.A.; Ansari, M.I.; Fujita, M.; Hasanuzzaman, M. Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms. *Antioxidants* **2021**, *10*, 277. <https://doi.org/10.3390/antiox10020277>.
13. Nile, S.H.; Thiruvengadam, M.; Wang, Y.; Samynathan, R.; Shariati, M.A.; Rebezov, M.; Nile, A.; Sun, M.; Venkidasamy, B.; Xiao, J.; et al. Nano-priming as emerging seed priming technology for sustainable agriculture—Recent developments and future perspectives. *J. Nanobiotechnol.* **2022**, *20*, 254. <https://doi.org/10.1186/s12951-022-01423-8>.
14. Gupta, K.J.; Fernie, A.R.; Kaiser, W.M.; Van Dongen, J.T. On the origins of nitric oxide. *Trends Plant Sci.* **2011**, *16*, 160–168. <https://doi.org/10.1016/j.tplants.2010.11.007>.
15. Baudouin, E.; Hancock, J.T. Nitric oxide signaling in plants. *Front. Plant Sci.* **2013**, *4*, 553. <https://doi.org/10.3389/fpls.2013.00553>.
16. Apel, K.; Hirt, H. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* **2004**, *55*, 373–399. <https://doi.org/10.1146/annurev.arplant.55.031903.141701>.
17. Mittler, R. ROS are good. *Trends Plant Sci.* **2017**, *22*, 11–19. <https://doi.org/10.1016/j.tplants.2016.08.002>.
18. Bykova, N.V.; Igamberdiev, A.U. Redox control of seed germination is mediated by the crosstalk of nitric oxide and reactive oxygen species. *Antioxid. Redox Signal.* **2025**, *42*, 442–461. <https://doi.org/10.1089/ars.2024.0699>.
19. Calabrese, E.J.; Mattson, M.P. How does hormesis impact biology, toxicology, and medicine? *npj Aging Mech. Dis.* **2017**, *3*, 13. <https://doi.org/10.1038/s41514-017-0013-z>.
20. Siemieniuk, A.; Rudnicka, M.; Jemioła, G.; Małkowski, E. Hormesis as a particular type of plant stress response. *Plants* **2025**, *14*, 3815. <https://doi.org/10.3390/plants14243815>.
21. Sánchez-Vicente, I.; Fátima Pollo-Rodríguez, F.; Mateos, I.; Huebra-Montero, L.; Corral-Barrio, V.; Albertos, P.; Lorenzo, O.; Mata-Pérez, C. Redox-mediated regulation of ABSCISIC ACID-INSENSITIVE 5 affects seed germination and seedling development in Arabidopsis. *J. Exp. Bot.* **2026**, erag006. <https://doi.org/10.1093/jxb/erag006>.
22. Rotondo, P.R.; Aceto, D.; Ambrico, M.; Stellacci, A.M.; Faretra, F.; De Miccolis Angelini, R.M.; Ambrico, P.F. Physicochemical properties of plasma-activated water and associated antimicrobial activity against fungi and bacteria. *Sci. Rep.* **2025**, *15*, 5536. <https://doi.org/10.1038/s41598-025-88369-7>.
23. Ongrak, P.; Poolyarat, N.; Harakotr, B.; Jirakiattikul, Y.; Rithichai, P. Plasma-activated water (PAW) enhances seed germination and improves biochemical quality in kangkong (*Ipomoea aquatica* Forssk.) microgreens. *Horticulturae* **2025**, *11*, 1218. <https://doi.org/10.3390/horticulturae11101218>.
24. Asghari, A.; Sabbaghtazeh, E.; Milani, N.R.; Kouhi, M.; Maralani, A.A.; Gharbani, P.; Khiaban, A.S. Effects of plasma-activated water on germination and initial seedling growth of wheat. *PLoS ONE* **2025**, *20*, e0312008. <https://doi.org/10.1371/journal.pone.0312008>.
25. Okruhlicová, Z.; Lukáčová, Z.; Hensel, K. Optimising plasma-activated water applications for enhanced growth and antioxidant capacity in maize hybrids: A comparative study of kernel priming, cultivation, and foliar application. *J. Phys. D Appl. Phys.* **2025**, *58*, 225202. <https://doi.org/10.1088/1361-6463/adc7c5>.
26. Guragain, R.P.; Baniya, H.B.; Banset, N.; Pradhan, S.P.; Dhungana, S.; Chhetri, G.P.; Panta, G.P.; Sedhai, B.; Shrestha, B.; Shrestha, S.; et al. Effects of plasma-activated water on soybean and wheat: Germination and seedling development. *Plasma Med.* **2022**, *12*, 27–43. <https://doi.org/10.1615/PlasmaMed.2022042374>.
27. Locatelli, S.; Triolone, S.; De Bonis, M.; Zanin, G.; Nicoletto, C. Non-thermal plasma-activated water enhances nursery production of vegetables: A species-specific study. *Agronomy* **2025**, *15*, 209. <https://doi.org/10.3390/agronomy15010209>.
28. Song, I.; Hong, S.; Na, Y.J.; Jang, S.Y.; Jung, J.Y.; Lee, Y.K.; Huh, S.U. Exploring the effects of diluted plasma-activated water (PAW) on various sprout crops and its role in autophagy regulation. *Agronomy* **2026**, *16*, 207. <https://doi.org/10.3390/agronomy16020207>.

29. Fan, L.; Liu, X.; Ma, Y.; Xiang, Q. Effects of plasma-activated water treatment on seed germination and growth of mung bean sprouts. *J. Taibah Univ. Sci.* **2020**, *14*, 823–830. <https://doi.org/10.1080/16583655.2020.1778326>.
30. Than, H.A.Q.; Pham, T.H.; Nguyen, D.K.V.; Pham, T.H.; Khacef, A. Non-thermal plasma activated water for increasing germination and plant growth of *Lactuca sativa* L. *Plasma Chem. Plasma Process.* **2021**, *42*, 73–89. <https://doi.org/10.1007/s11090-021-10210-6>.
31. Fatelnig, L.M.M.; Chanyalew, S.; Tadesse, M.; Kebede, W.; Hussein, N.; Iza, F.; Tadele, Z.; Leubner-Metzger, G.; Steinbrecher, T. Seed priming with gas plasma-activated water in Ethiopia's "orphan" crop tef (*Eragrostis tef*). *Planta* **2024**, *259*, 75. <https://doi.org/10.1007/s00425-024-04359-5>.
32. Kostoláni, D.; Ndiffo Yemeli, G.B.; Švubová, R.; Kyzek, S.; Machala, Z. Physiological responses of young pea and barley seedlings to plasma-activated water. *Plants* **2021**, *10*, 1750. <https://doi.org/10.3390/plants10081750>.
33. Hou, C.Y.; Kong, T.K.; Lin, C.M.; Chen, H.L. The effects of plasma-activated water on heavy metals accumulation in water spinach. *Appl. Sci.* **2021**, *11*, 5304. <https://doi.org/10.3390/app11115304>.
34. Chuea-uan, S.; Boonyawan, D.; Sawangrat, C.; Thanapornpoonpong, S.N. Using plasma-activated water generated by an air gliding arc as a nitrogen source for rice seed germination. *Agronomy* **2024**, *14*, 15. <https://doi.org/10.3390/agronomy14010015>.
35. International Seed Testing Association (ISTA). *International Rules for Seed Testing*; ISTA: Wallisellen, Switzerland, 2025.
36. Geneve, R.L. Vigour testing in flower seeds. In *Flower Seeds: Biology and Technology*; McDonald, M.B., Kwong, F.Y., Eds.; CABI Publishing: Wallingford, UK, 2005; pp. 311–332.
37. Maguire, J.D. Speed of germination-aid in selection and evaluation for seedling emergence and vigor. *Crop Sci.* **1962**, *2*, 176–177. <https://doi.org/10.2135/cropsci1962.0011183X000200020033x>.
38. Bissoli, G.; Bono, M.; Martínez-Almonacid, I.; Moreno-Peris, E.; Renard, J.; Espinosa, A.; Naranjo, M.Á.; Yenush, L.; Fita, A.; Serrano, R.; et al. Seed coat lignification level is crucial in *Capsicum* spp. seed longevity. *Physiol. Plant.* **2022**, *174*, e13600. <https://doi.org/10.1111/ppl.13600>.
39. Billah, M.; Sajib, S.A.; Roy, N.C.; Rashid, M.M.; Rwza, M.A.; Hasan, M.M.; Talukder, M.R. Effects of DBD air plasma treatment on the enhancement of black gram (*Vigna mungo* L.) seed germination and growth. *Arch. Biochem. Biophys.* **2020**, *681*, 108253. <https://doi.org/10.1016/j.abb.2020.108253>.
40. Hasanuzzaman, M.; Bhuyan, M.H.M.B.; Zulfiqar, F.; Raza, A.; Mohsin, S.M.; Mahmud, J.A.; Fujita, M.; Fotopoulos, V. Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. *Antioxidants* **2020**, *9*, 681. <https://doi.org/10.3390/antiox9080681>.
41. da Silva, L.J.; Dias, D.C.F.D.S.; Sekita, M.C.; Finger, F.L. Lipid peroxidation and antioxidant enzymes of *Jatropha curcas* L. seeds stored at different maturity stages. *Acta Sci. Agron.* **2018**, *40*, e34978. <https://doi.org/10.4025/actasciagron.v40i1.34978>.
42. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid determination of free proline for water-stress studies. *Plant Soil* **1973**, *39*, 205–207. <https://doi.org/10.1007/BF00018060>.
43. Önder, S.; Önder, D.G.; Tonguç, M. Determination of hydrogen peroxide content and antioxidant enzyme activities in safflower (*Carthamus tinctorius* L.) seeds after accelerated aging test. *J. Nat. Appl. Sci.* **2020**, *24*, 681–688.
44. Heshmati, S.; Dehaghi, M.A.; Farooq, M.; Wojtyła, Ł.; Maleki, K.; Heshmati, S. Role of melatonin seed priming on antioxidant enzymes and biochemical responses of *Carthamus tinctorius* L. under drought stress conditions. *Plant Stress* **2021**, *2*, 100023. <https://doi.org/10.1016/j.stress.2021.100023>.
45. Zhang, S.Z.; Hua, B.Z.; Zhang, F. Induction of the activities of antioxidative enzymes and the levels of malondialdehyde in cucumber seedlings as a consequence of *Bemisia tabaci* (Hemiptera: Aleyrodidae) infestation. *Arthropod Plant Interact.* **2008**, *2*, 209–213. <https://doi.org/10.1007/s11829-008-9044-5>.
46. McCleary, B.V.; McNally, M.; Monaghan, D.; Mugford, D.C. Measurement of α -amylase activity in white wheat flour, milled malt, and microbial enzyme preparations, using the Ceralpha assay: Collaborative study. *J. AOAC Int.* **2002**, *85*, 1096–1102. <https://doi.org/10.1093/jaoac/85.5.1096>.
47. DuBois, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350–356. <https://doi.org/10.1021/ac60111a017>.
48. Bruggeman, P.J.; Kushner, M.J.; Locke, B.R.; Gardeniers, J.G.E.; Graham, W.G.; Graves, D.B.; Hofman-Caris, R.C.H.M.; Maric, D.; Reid, J.P.; Ceriani, E.; et al. Plasma–liquid interactions: A review and roadmap. *Plasma Sources Sci. Technol.* **2016**, *25*, 053002. <https://doi.org/10.1088/0963-0252/25/5/053002>.
49. Montalbetti, R.; Machala, Z.; Gherardi, M.; Laurita, R. Production and chemical composition of plasma-activated water: A systematic review and meta-analysis. *Plasma Process. Polym.* **2024**, *22*, 2400249. <https://doi.org/10.1002/ppap.202400249>.

50. Stapelmann, K.; Gershman, S.; Miller, V. Plasma–liquid interactions in the presence of organic matter: A perspective. *J. Appl. Phys.* **2024**, *135*, 160901. <https://doi.org/10.1063/5.0203125>.
51. Machala, Z.; Tarabova, B.; Hensel, K.; Špetlíková, E.; Šikurová, L.; Lukeš, P. Formation of ROS and RNS in water electro-sprayed through transient spark discharge in air and their bactericidal effects. *Plasma Process. Polym.* **2013**, *10*, 649–659.
52. Lee, G.; Choi, S.; Yoo, M.; Chang, H.; Lee, N. Effects of plasma-activated water treatment on the inactivation of microorganisms present on cherry tomatoes and in used wash solution. *Foods* **2023**, *12*, 2461. <https://doi.org/10.3390/foods12132461>.
53. Acker, K.; Möller, D.; Auel, R.; Wieprecht, W.; Kalaß, D. Concentrations of nitrous acid, nitric acid, nitrite and nitrate in the gas and aerosol phase at a site in the emission zone during ESCOMPTE 2001 experiment. *Atmos. Res.* **2004**, *74*, 507–524. <https://doi.org/10.1016/j.atmosres.2004.04.009>.
54. Rahman, M.; Hasan, M.S.; Islam, R.; Rana, R.; Sayem, A.; Sad, M.A.A.; Matin, A.; Raposo, A.; Zandonadi, R.P.; Han, H.; et al. Plasma-activated water for food safety and quality: A review of recent developments. *Int. J. Environ. Res. Public Health* **2022**, *19*, 6630. <https://doi.org/10.3390/ijerph19116630>.
55. Bienert, G.P.; Möller, A.L.B.; Kristiansen, K.A.; Schulz, A.; Möller, I.M.; Schjoerring, J.K.; Jahn, T.P. Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. *J. Biol. Chem.* **2007**, *282*, 1183–1192. <https://doi.org/10.1074/jbc.M603761200>.
56. Varadaraj, K.; Kumari, S.S. Lens aquaporins function as peroxiporins to facilitate membrane transport of hydrogen peroxide. *Biochem. Biophys. Res. Commun.* **2020**, *524*, 1025–1029. <https://doi.org/10.1016/j.bbrc.2020.02.031>.
57. Wang, Y.; Zhao, Z.; Liu, F.; Sun, L.; Hao, F. Versatile roles of aquaporins in plant growth and development. *Int. J. Mol. Sci.* **2020**, *21*, 9485. <https://doi.org/10.3390/ijms21249485>.
58. Zhao, Z.; Li, M.; Xu, W.; Liu, J.; Li, C. Genome-wide identification of NRT gene family and expression analysis of nitrate transporters in response to salt stress in *Poncirus trifoliata*. *Genes* **2022**, *13*, 1115. <https://doi.org/10.3390/genes13071115>.
59. Khan, M.; Ali, S.; Al Azzawi, T.N.I.; Saqib, S.; Ullah, F.; Ayaz, A.; Zaman, W. The key roles of ROS and RNS as signaling molecules in plant–microbe interactions. *Antioxidants* **2023**, *12*, 268. <https://doi.org/10.3390/antiox12020268>.
60. Duan, W.; Wang, S.; Zhang, H.; Xie, B.; Zhang, L. Plant growth and nitrate absorption and assimilation of two sweet potato cultivars with different nitrogen tolerances in response to nitrate supply. *Sci. Rep.* **2024**, *14*, 21286. <https://doi.org/10.1038/s41598-024-72422-y>.
61. Arc, E.; Galland, M.; Godin, B.; Cueff, G.; Rajjou, L. Nitric oxide implication in the control of seed dormancy and germination. *Front. Plant Sci.* **2013**, *4*, 346. <https://doi.org/10.3389/fpls.2013.00346>.
62. Singh, R.; Singh, S.; Parihar, P.; Mishra, R.K.; Tripathi, D.K.; Singh, V.P.; Chauhan, D.K.; Prasad, S.M. Reactive oxygen species (ROS): Beneficial companions of plant developmental processes. *Front. Plant Sci.* **2016**, *7*, 1299. <https://doi.org/10.3389/fpls.2016.01299>.
63. Talaat, N.B. Role of reactive oxygen species signaling in plant growth and development. In *Reactive Oxygen, Nitrogen and Sulfur Species in Plants: Production, Metabolism, Signaling and Defense Mechanisms*; Hasanuzzaman, M., Fotopoulos, V., Nahar, K., Fujita, M., Eds.; Wiley-Blackwell: Hoboken, NJ, USA, 2019; pp. 225–266.
64. Ali, S.; Tyagi, A.; Bae, H. ROS interplay between plant growth and stress biology: Challenges and future perspectives. *Plant Physiol. Biochem.* **2023**, *203*, 108032. <https://doi.org/10.1016/j.plaphy.2023.108032>.
65. Sharma, P.; Jha, A.B.; Dubey, R.S.; Pessarakli, M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J. Bot.* **2012**, *2012*, 217037. <https://doi.org/10.1155/2012/217037>.
66. Zaboli, M.; Nejad-Alimoradi, F.; Rostami, F.; Keramat, B.; Mousavi, E.A.; Nasibi, F. Seed priming and irrigating with plasma-activated water improve the growth and drought resistance in *Poa pratensis*. *BMC Plant Biol.* **2025**, *25*, 1747. <https://doi.org/10.1186/s12870-025-07742-w>.
67. Xie, X.; He, Z.; Chen, N.; Tang, Z.; Wang, Q.; Cai, Y. The roles of environmental factors in regulation of oxidative stress in plants. *Biomed Res. Int.* **2019**, *2019*, 9732325. <https://doi.org/10.1155/2019/9732325>.
68. Qamer, Z.; Chaudhary, M.T.; Du, X.; Hinze, L.; Azhar, M.T. Review of oxidative stress and antioxidative defense mechanisms in *Gossypium hirsutum* L. in response to extreme abiotic conditions. *J. Cotton Res.* **2021**, *4*, 9. <https://doi.org/10.1186/s42397-021-00086-4>.
69. Souza, J.M.; Choi, I.; Chen, Q.; Weisse, M.; Daikhin, E.; Yudkoff, M.; Obin, M.; Ara, J.; Horwitz, J.; Ischiropoulos, H. Proteolytic degradation of tyrosine nitrated proteins. *Arch. Biochem. Biophys.* **2000**, *380*, 360–366. <https://doi.org/10.1006/abbi.2000.1940>.
70. Cabiscol, E.; Tamarit, J.; Ros, J. Protein carbonylation: Proteomics, specificity and relevance to aging. *Mass Spectrom. Rev.* **2014**, *33*, 21–48. <https://doi.org/10.1002/mas.21375>.

71. Li, B.; Zhang, S.; Lv, Y.; Wei, S.; Hu, Y. Reactive oxygen species-induced protein carbonylation promotes deterioration of physiological activity of wheat seeds. *PLoS ONE* **2022**, *17*, e0263553. <https://doi.org/10.1371/journal.pone.0263553>.
72. Beligni, M.V.; Fath, A.; Bethke, P.C.; Lamattina, L.; Jones, R.L. Nitric oxide acts as an antioxidant and delays programmed cell death in barley aleurone layers. *Plant Physiol.* **2002**, *129*, 1642–1650. <https://doi.org/10.1104/pp.002337>.
73. Nonogaki, H.; Bassel, G.W.; Bewley, J.D. Germination—Still a mystery. *Plant Sci.* **2010**, *179*, 574–581. <https://doi.org/10.1016/j.plantsci.2010.02.010>.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.