



Cellular and Molecular Mechanisms of Cold Plasma Action Under Pathogen-Associated Salinity Stress *In-Vitro* Study



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Abstract

Background: Salinity is a major abiotic stress that severely limits the growth and productivity of sweet pepper (*Capsicum annuum* L.) by inducing osmotic imbalance, ionic toxicity, and oxidative stress. Developing sustainable and eco-friendly approaches to enhance plant tolerance under saline conditions is therefore essential. **Objectives:** This study aimed to investigate the effectiveness of cold plasma (dielectric barrier discharge device, DBD) seed priming in improving germination, growth performance, biochemical responses, and stress-related gene expression in sweet pepper under pathogen-associated salinity stress. **Methodology:** This experimental study was conducted in the Department of Biology, College of Science for Women, University of Baghdad, Baghdad, Iraq. Between December 2024 to September 2025. Seeds were treated with cold plasma for 60 and 180 seconds and cultured under different salinity levels (8, 10, and 12 dS/m). Germination parameters and seedling growth traits were evaluated, along with callus induction and growth. Biochemical analyses included proline content and antioxidant enzymes (CAT and SOD), while gene expression of CaDHN3 and CaSOS1 was quantified using qRT-PCR. **Results:** Plasma treatment significantly improved germination percentage (from 75% to 91.67%) and enhanced seedling growth, particularly root development (25.33 roots at 8 dS/m). Salinity reduced growth and callus biomass; however, plasma mitigated these effects under moderate stress. Biochemical responses showed increased proline accumulation (41.06 $\mu\text{mol/g}$ FW) and enhanced antioxidant enzyme activities, with CAT reaching 0.976 U/mL and SOD 145.28 U/mL. At the molecular level, CaDHN3 expression increased up to 22.47-fold in callus and 13.74-fold in leaves, while CaSOS1 reached 13.64-fold and 13.18-fold, respectively, indicating improved osmotic adjustment and ion homeostasis. **Conclusion:** Cold plasma priming effectively enhances physiological, biochemical, and molecular defense mechanisms in sweet pepper under salinity stress. This approach improves germination, growth, antioxidant capacity, and stress-responsive gene expression, offering a promising and sustainable strategy for enhancing salinity tolerance and crop productivity. [*Bangladesh Journal of Infectious Diseases*, June 2026;13(1):113-125]

Keywords: *Capsicum annuum* L.; Pathogen; Cold plasma; Callus culture; Gene expression; Salinity stress

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Introduction

Salinity represents a major threat to global agriculture, as most plants are unable to tolerate high salt concentrations. It adversely affects plant growth and productivity through morphological,

physiological, and biochemical disruptions, including reduced germination, impaired photosynthesis, nutrient imbalance, membrane damage, and ultimately decreased yield and quality¹. These effects are primarily associated with

excessive accumulation of Na^+ and Cl^- ions, which interfere with the uptake of essential nutrients such as K^+ and Ca^{2+} , leading to osmotic stress, ionic imbalance, and oxidative damage caused by reactive oxygen species (ROS)^{2,3}.

Sweet pepper (*Capsicum annuum* L.), a nutritionally and economically valuable crop of the Solanaceae family, is widely cultivated and appreciated for its high content of vitamins, minerals, and bioactive compounds. However, it is moderately sensitive to salinity, and its productivity is significantly constrained under saline conditions, necessitating the development of effective and sustainable strategies to enhance stress tolerance⁴. Cold plasma technology, particularly the dielectric barrier discharge (DBD) device, has emerged as a promising, environmentally friendly approach in agriculture. This non-thermal technology generates reactive oxygen and nitrogen species (RONS), along with charged particles and UV radiation, without causing thermal damage to plant tissues. These reactive components can induce physiological and molecular modifications, including improved seed surface properties, enhanced osmolyte accumulation such as proline, strengthened antioxidant defense systems, and improved ion homeostasis, thereby promoting plant performance under salinity stress^{5,6}.

Plants respond to salinity through various adaptive mechanisms, including the induction of stress-responsive proteins such as dehydrins (DHNs), which belong to the LEA protein family. These proteins play a crucial role in maintaining cellular stability, scavenging ROS, and regulating osmotic balance under stress conditions. In particular, the *CaDHN3* gene has been associated with enhanced salinity tolerance through cellular protection, promoting germination and root growth in vitro and improving antioxidant activity, whereas its suppression leads to increased oxidative damage and reduced stress tolerance^{7,8}. Another key mechanism in salinity tolerance is the Salt Overly Sensitive (SOS) pathway, which regulates ion homeostasis through the coordinated action of SOS1, SOS2, and SOS3 proteins. SOS1 functions as a plasma membrane Na^+/H^+ antiporter that facilitates sodium extrusion, thereby reducing ionic toxicity and maintaining the K^+/Na^+ balance essential for cellular processes. Evidence from several crops indicates that SOS1 plays a critical role in enhancing salt tolerance, and *CaSOS1* is believed to perform a similar function in sweet pepper^{9,10}.

Therefore, this study was aimed to evaluate the role

of cold plasma (DBD) treatment in improving salinity tolerance in *Capsicum annuum*. Specifically, it investigates its effects on early growth performance, key biochemical indicators such as proline and antioxidant enzymes (SOD and CAT), and the expression of stress-related genes (*CaDHN3* and *CaSOS1*) in both leaf and callus tissues under salinity conditions.

Methodology

Study Design and Settings: This was a laboratory-based experimental study. This study was conducted in the Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq from December 2024 to September 2025.

Study Procedure: Seed treatment via Atmospheric Cold Plasma Dielectric Barrier Discharge (DBD) device was applied. In brief, sweet pepper seeds (*Capsicum annuum* L., Kamar F1) were obtained from Debbane Agriculture Holding (Baghdad, Iraq) and taxonomically verified at the herbarium of the University of Baghdad. Seed treatment was conducted using an atmospheric cold plasma dielectric barrier discharge (DBD) device in the Plasma Laboratory, Department of Physics, College of Science for Women, University of Baghdad. The DBD system consisted of two parallel stainless-steel electrodes (90 mm diameter) separated by a quartz dielectric barrier and insulated with a 10 mm Teflon layer. Plasma was generated using a high-voltage alternating current (~15 kV) to ionize ambient air and produce non-thermal plasma. Seeds were placed at a distance of 2-3 mm from the electrode surface and exposed to plasma for 60 and 180 seconds to ensure effective interaction¹¹ (Figure I).



Figure I: Atmospheric Cold Plasma Dielectric Barrier Discharge (DBD) Device

Seed Germination and Seedling Growth under Salinity Conditions: Sweet pepper seeds were surface sterilized in the plant tissue culture

Germination speed (GSP) (%) = $\frac{\text{Total number of seeds germinated at 24 h for 7 days}}{\text{Total number of seeds in all replicates}} \times 100$	No. 1
Germination percentage (GP) (%) = $\frac{\text{Number of germinated seeds}}{\text{Total number of cultured seeds}} \times 100$	No. 2

laboratory (Department of Biology, College of Science for Women, University of Baghdad) using 70.0% ethanol for 1 minute followed by 2% sodium hypochlorite (NaOCl) for 15 min, then rinsed three times with sterile distilled water under aseptic conditions in a laminar airflow cabinet. Sterilized seeds were subsequently exposed to cold plasma for 60 and 180 sec as previously described¹², then cultured on Murashige and Skoog (MS) medium. The experiment was arranged in three replicates (12 seeds per treatment) and incubated at $25 \pm 2^\circ\text{C}$ under a 16/8 h light/dark photoperiod (1000 lux). Germination percentage and rate were recorded after 7 days using standard equation no. 1 and equation no. 2¹³, while seedling growth parameters (shoot and root lengths, leaf and root numbers) were measured after 30 days.

To evaluate salinity effects, treated seeds were cultured on MS medium supplemented with different NaCl concentrations. The control medium had an electrical conductivity (EC) of 5 dS/m, while salinity treatments were adjusted to 8, 10, and 12 dS/m. EC was measured using a conductivity meter. Growth conditions were maintained as described above, and both germination traits (after 7 days) and seedling growth characteristics (after 30 days) were assessed¹⁴.

Callus Induction and Growth under Salinity Conditions: Leaf and stem explants of *Capsicum annum* were cultured on Murashige and Skoog (MS) medium supplemented with 0.5 mg/L naphthalene acetic acid (NAA) and 1.0 mg/L kinetin (KIN) to induce callus formation¹⁵. Cultures were incubated at $25.0 \pm 2.00^\circ\text{C}$ under a 16/8 h light/dark photoperiod (1000 lux). After four weeks,

callus induction percentage was recorded, and the induction frequency was determined using equation 3 reported by¹⁶. Fresh weight was measured as the mean of ten replicates. Calli derived from leaf explants were transferred to MS medium containing the same growth regulators and different NaCl concentrations. Salinity levels were adjusted to 8, 10, and 12 dS/m, while the control medium had an EC of 5 dS/m. Approximately 100 mg of callus tissue per treatment was cultured under the same environmental conditions. After four weeks, callus fresh weight was recorded to evaluate the effect of salinity¹⁷.

Biochemical Analysis of Callus under Salinity Stress: Proline content in callus tissues was determined following the method of⁸. Fresh callus samples (0.5 g) were extracted with 3% sulfosalicylic acid, and the filtrate was reacted with acid ninhydrin and glacial acetic acid, incubated at 100°C for 1 h, then cooled. The chromophore was extracted with toluene, and absorbance was measured at 520 nm. Proline concentration was calculated using a standard curve and expressed as $\mu\text{mol/g}$ fresh weight. Antioxidant enzyme activities, including catalase (CAT) and superoxide dismutase (SOD), were also assessed in callus grown under salinity. CAT activity was measured spectrophotometrically by monitoring H_2O_2 decomposition at 240 nm using enzyme extracts prepared in phosphate buffer¹⁹. SOD activity was determined based on inhibition of pyrogallol auto-oxidation in Tris-EDTA buffer (pH 8.2), and expressed as the amount of enzyme required to achieve 50.0% inhibition²⁰, calculate using the formulas provided in equations (4) and (5).

Callus induction Percentage from explants = $\frac{\text{No. of explants producing callus}}{\text{No. of cultured explants}} \times 100$	No. 3
SOD Activity (Unit/mL) = $\frac{\% \text{ Inhibition of pyrogallol auto-oxidant}}{\% 50}$	No. 4
Inhibition of pyrogallol auto -oxidant (%) = $\frac{(\Delta A_{\text{Control}}) - (\Delta A_{\text{Test}})}{(\Delta A_{\text{Control}})} \times 100$	No. 5

Table 1: Sequences of the Primers for Gene Expression

Name of Gene	Primer	Primer Sequence(5'→3')	Reference
CaDHN3	Forward	ATGGCACATAACGGTACTAGCC	Jing et al23
	Reverse	CCCTTCATCTTTCTTCATAGCAT	
CaSOS1	Forward	GTTCGTGTCTCGTTTCCGC	Mravljje et al24
	Reverse	TCAAATCGGTCTGAACAGCATC	
CaActin2	Forward	TCCACCTCTTCACTCTCTGCTC	
	Reverse	TGACCCATCCCTACCATAACAC	

RNA Extraction and Gene Expression Analysis

(qRT-PCR): Total RNA was extracted from leaf and callus tissues using TRIzol™ Reagent (Invitrogen, USA) according to the manufacturer's protocol²¹. Samples (100 mg) were homogenized, then phase-separated with chloroform, RNA precipitated with isopropanol, washed with 75% ethanol, and dissolved in RNase-free water. RNA concentration and purity were determined using a Qubit 4 (ThermoFisher®, USA) fluorometer. Complementary DNA (cDNA) was synthesized from total RNA using the ProtoScript® First Strand cDNA Synthesis Kit (New England Biolabs, UK), which contains M-MuLV Reverse Transcriptase and an RNase inhibitor under standard reaction conditions²². Gene expression analysis was performed using SYBR Green-based qRT-PCR, targeting *CaDHN3* and *CaSOS1* genes, with *CaActin2* as an internal reference. All primers were synthesized by Macrogen (Korea) and diluted to working concentrations of 10 µM. The name and sequence are given in Table 1. Reactions were conducted in a 20 µL volume using Luna® Universal qPCR Master Mix (NEB), and amplification was performed using an initial denaturation at 95°C for 60 sec, followed by 40-45 cycles of denaturation at 95°C for 30 sec, annealing at 58°C for 30 sec, and extension at 72°C for 90 sec. A final melt curve analysis (60–95°C) was conducted to confirm amplification specificity. Gene expression analysis employed the $2^{-\Delta\Delta Ct}$ method²⁵. ΔCt values were computed by subtracting the Ct of the housekeeping gene from that of the target gene. The $\Delta\Delta Ct$ was derived by comparing experimental samples to control samples. Fold changes in gene expression were then calculated as $2^{-\Delta\Delta Ct}$, with results expressed as relative ratios of experimental groups to control.

Statistical Analyses: Data were analyzed using the Social Sciences program (SPSS)26 Version 26.0 (IBM, Armonk, NY). ANOVA with LSD test was employed to compare means, while chi-square tests evaluated percentage differences at significance levels of 0.05 and 0.01. For gene expression

analysis, non-parametric tests, specifically Kruskal–Wallis and Mann–Whitney, were used to compare treatment groups with controls.

Ethical Consideration: The plant experimental study was conducted in accordance with relevant institutional and environmental guidelines. All plant materials were collected, handled, and used responsibly to minimize unnecessary damage to the environment and to ensure sustainable use of resources. Appropriate permissions were obtained where required for sample collection, and care was taken to avoid disturbance to surrounding habitats. The study was designed to use the minimum quantity of plant material necessary to achieve valid results, and all waste materials were disposed of properly in accordance with laboratory and environmental safety procedures.

Results**Influence of DBD Plasma Device Treatment on the Germination Speed and Percentage under Salinity Conditions:**

As presented in Figure II, seeds exposed to cold plasma generated by a dielectric barrier discharge (DBD) device for two exposure times (60 and 180 sec) showed significantly ($P < 0.01$) higher germination speed (GSP) and germination percentage (GP) compared with untreated seeds (control). From days 7 to 13 post-planting, we observed an evident change in germination speed in (Figure IIa), the seeds with 180 seconds of exposure had the fastest germination speed; those with 60 seconds of exposure were next; untreated controls were consistently lowest rates. Differences were found to be statistically significant ($P \leq 0.01$). Similarly, germination percentages in (Figure IIb), treated seeds had statistically higher germination percentages than untreated seeds. On the 13th day, seeds exposed to 180 seconds of plasma treatment achieved the highest germination percentage (91.67%), while those exposed to 60 seconds followed (83.3%), in comparison to untreated seeds, which were severely behind (75.0%), clearly indicating that the cold plasma (DBD) treatments play an important role in

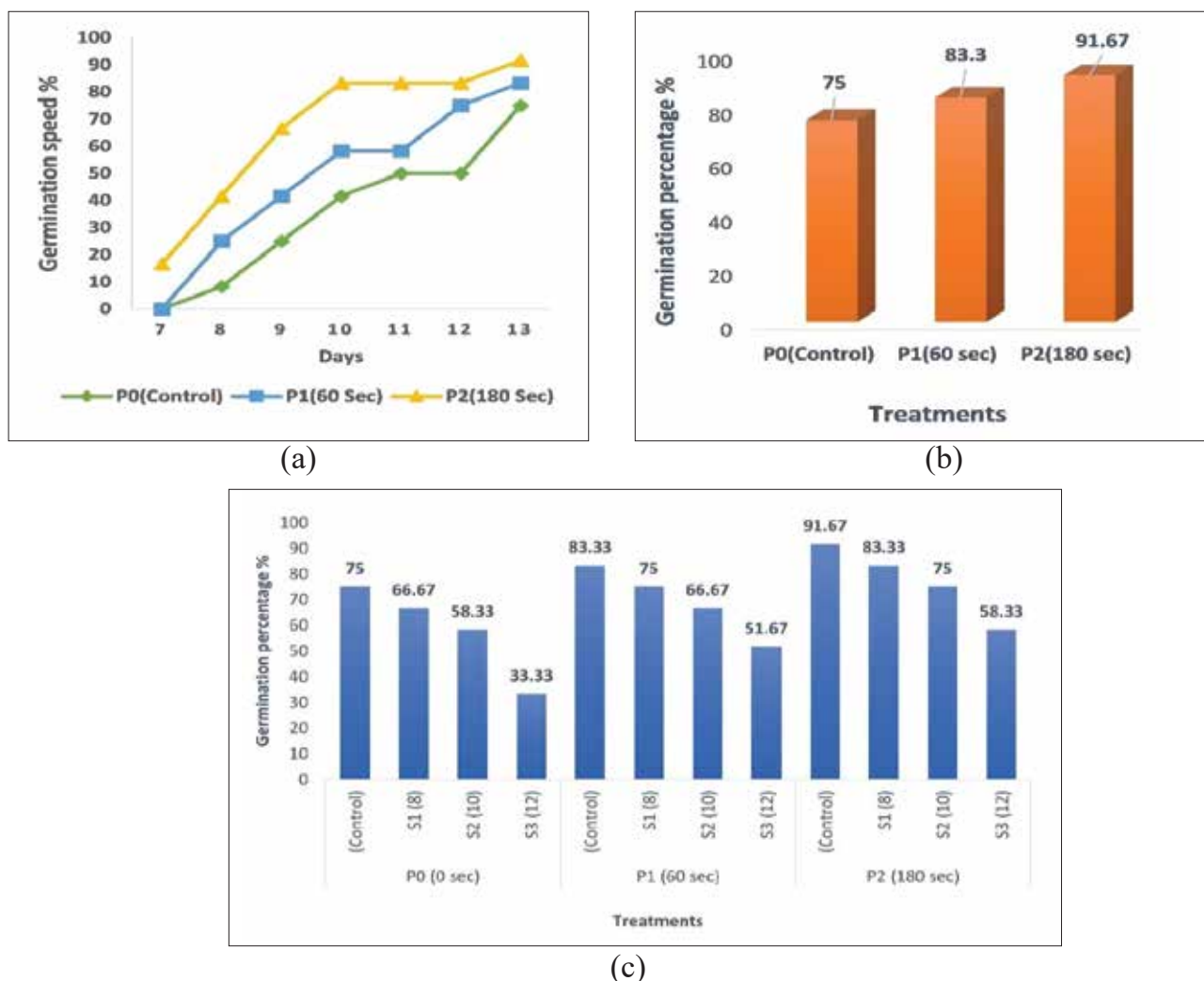


Figure II: Effects of Cold Plasma (DBD) Treatment on Sweet Pepper Seed on (a) Germination Speed (GSP) and (b) Germination Percentage (GP), (c) Germination Percentage of Seeds

accelerating germination of sweet pepper seeds. Salinity stress significantly reduced GP, particularly at 12 ds/m, where untreated seeds recorded the lowest germination (33.33%) (Figure IIC). However, plasma treatment mitigated these adverse effects, as seeds exposed to 180 sec maintained higher germination percentage even under elevated salinity levels.

Morphological Traits of Sweet Pepper Seedlings under Salinity Conditions: Cold plasma (DBD) treatment significantly improved the morphological traits of sweet pepper seedlings compared with the control (Table 2). Seedlings treated for 180 sec showed the greatest shoot length (4.97 cm) compared to 4.13 cm in untreated plants. Although leaf number showed a slight increase, differences were not statistically significant. In contrast, root development was markedly enhanced, with plasma-treated seedlings producing 16.7 roots compared to 10.3 in the control. Salinity (8, 10, and

12 ds/m) significantly reduced growth parameters, particularly shoot length, which declined with increasing salt concentration, as shown in (Figure III). However, plasma treatment, especially at 180 sec, mitigated these adverse effects and maintained higher growth under saline conditions. At 8 ds/m, treated seedlings exhibited superior root traits, reaching 25.33 roots and 8.67 cm in root length compared to untreated plants.

Stimulation and growth of callus exposed to cold plasma under Salinity conditions: Cold plasma (DBD) treatment significantly influenced callus induction and growth of *Capsicum annum* explants in combination with plant growth regulators (NAA + KIN). The highest induction frequencies were obtained at 60 sec exposure, reaching 100.0% in leaf explants and 90.9% in stem explants (Figure IVa), with the formation of compact, yellowish callus, indicating effective dedifferentiation under balanced auxin–cytokinin conditions. A comparable

Table 2: Effects of Cold Plasma (DBD) Treatment on Sweet Pepper Seeds Cultivated in Media Containing Different NaCl Concentrations on Shoot and Root Length (Cm), and Number of Leaves & Roots

Exposure time (sec)	Salt concentration (dS/m)	Shoot length (cm)	Number of leaves	Root length (cm)	Number of Roots
P0 Control	(Control)	4.133 ±0.17 ab	7 ±0.22	5 ±0.10 b	10.33 ±0.64 d
	S1 (8)	3.833 ±0.05 ab	7 ±0.22	4.433 ±0.16 b	20.33 ±1.26 bc
	S2 (10)	3.50 ±0.05 b	6 ±0.18	4.033 ±0.17 b	19.33 ±1.05 bc
	S3 (12)	3.10±0.05b	4.8±1.18	3.91±0.18 b	18.33±1.05 bc
P1 (60 sec)	(Control)	4.433 ±0.19 ab	6 ±0.18	5.233 ±0.24 b	16.33 ±0.81 c
	S1 (8)	4.367 ±0.18 ab	7 ±0.22	6.533 ±0.27 ab	25.33 ±2.37 a
	S2 (10)	3.867 ±0.14 ab	6 ±0.18	5.367 ±0.24 b	22.67 ±1.08 ab
	S3 (12)	3.36±0.14 b	5±0.13	4.45±0.34 b	19.7±1.65 bc
P2 (180 sec)	(Control)	4.967 ±0.18 a	7 ±0.22	5.133 ±0.16 b	16.67 ±0.76 c
	S1 (8)	4.733 ±0.16 ab	6 ±0.18	8.667 ±0.47 a	23.67 ±1.63 ab
	S2 (10)	4.567 ±0.22 ab	6 ±0.18	4.567 ±0.19 b	22.33 ±1.27 ab
	S3 (12)	4.15±0.54 ab	5±0.18	4.16±0.19 b	20.3±1.63 bc
L.S.D.		1.271 *	1.06 NS	2.265 *	5.017 *

Data are expressed as means ±SD (n =10). Different letters following the data within each column mean a significant difference at * P≤0.05. Abbreviations: P, Plasma treatment; S, Salinity level

Cultivated in Media Containing Different NaCl Concentrations



Figure III: Showing Effects of Cold Plasma Seed Treatment and NaCl Concentrations on the Morphology of Sweet Pepper Seedlings After 30 Days of Germination Grown in Hormone-Free MS Medium. (A-C) Untreated Seedlings: without Salt (A), with 8 dS/m NaCl (B), and with 10 dS/m NaCl (C). (D-F) Seedlings Treated with Plasma for 60 Sec: without Salt (D), with 8 dS/m NaCl (E), and with 10 dS/m NaCl (F). (G-I) Seedlings Treated with Plasma for 180 Sec: without Salt (G), with 8 dS/m NaCl (H), and with 10 dS/m NaCl (I), Plasma-Treated Seedlings Generally Exhibited Improved Root Development and Better Overall Growth Compared with Untreated Seedlings Under the Same Salinity Conditions

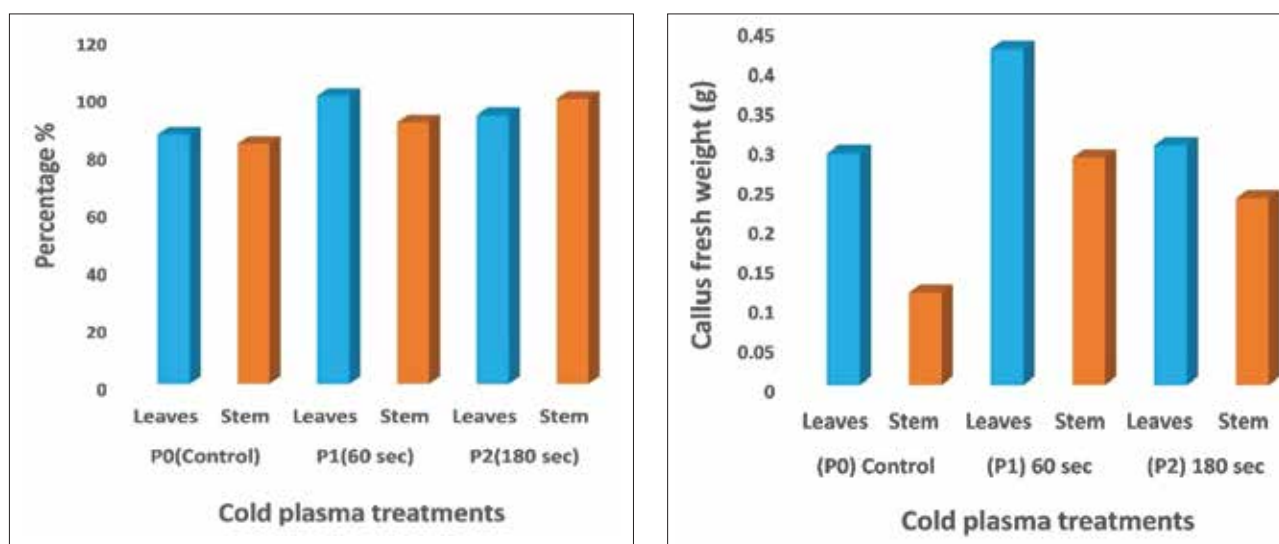


Figure IV: Effect of the Combination of 0.5 NAA mg/L and 1.0 KIN mg/L on (a) Callus Induction Percentage, and (b) Callus Fresh Weight from various Explants of *Capsicum annuum* L.

but slightly lower response was observed at 180 sec. Similarly, callus fresh weight was maximized at 60 sec (leaf: 0.4243 g; stem: 0.2872 g), as evident in (Figure IVb), while 180 sec produced lower

biomass. Salinity significantly reduced callus growth, as evidenced by the decline in fresh weight from 0.529 g (control) to 0.108 g at 12 dS/m (Table 3). However, plasma-treated calli (60 and 180 sec)

Table 3: Effect of Different NaCl Concentrations on Callus Fresh Weight (G) After Four Weeks of Cultivation on MS Medium With 0.5 NAA Mg/L And 1 KIN Mg/L. The Initial Weight Is 100 Mg

Exposure time (sec)	MS medium with 0.5 NAA mg/L + 1 KIN mg/L				L.S.D.
	Control	Salt (dS/m)			
		8	10	12	
P0 (Control)	0.529 ±0.11 a	0.425 ±0.04 a	0.345 ±0.04 ab	0.108 ±0.02 b	0.237 *
P1 (60 sec)	0.542 ±0.08 a	0.484 ±0.07 a	0.387 ±0.04 a	0.149 ±0.03 b	0.208 *
P2 (180 sec)	0.533 ±0.08 a	0.452 ±0.05 a	0.373 ±0.06 a	0.125 ±0.03 b	0.224 *
L.S.D.	0.107 NS	0.0673 NS	0.062 NS	0.055 NS	0.066 NS

Data are expressed as means ±SD (n=10). Different letters following the data within each row mean a significant difference at * P≤0.05, NS: Non-Significant. Abbreviation: P, plasma treatment.

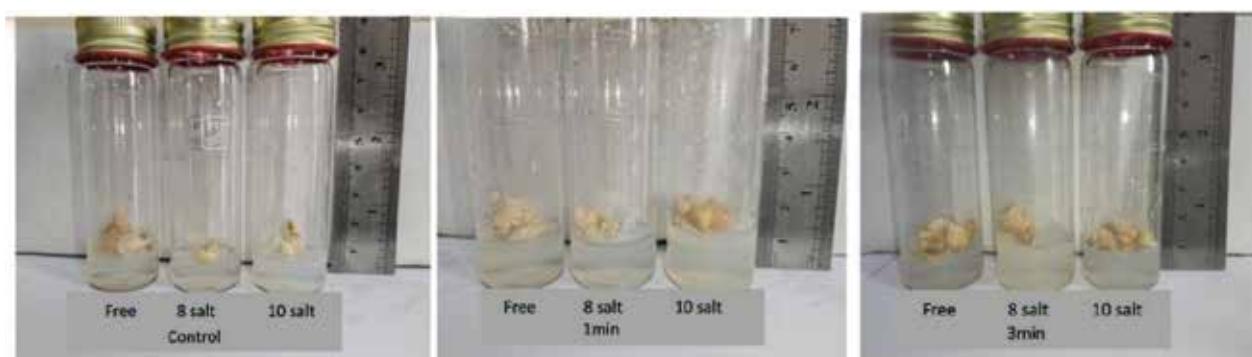


Figure V: Callus Induction from Sweet Pepper Leaves in MS Medium Supplemented With 0.5 Mg/L NAA and 1.0 Mg/L KIN, With NaCl Salinity Levels of 8 and 10 dS/m After Four Weeks of Culture. In (A) Untreated Seedlings with Plasma; (B) Seedlings Treated with Plasma For 60 Seconds; and (C) Seedlings Treated with Plasma For 180 Seconds

maintained relatively higher fresh weights under moderate salinity levels (8 and 10 dS/m), 60 sec treatment yielded 0.484 and 0.387 g, respectively, compared to 0.425 and 0.345 g in untreated calli (Figure V). At the highest salinity level (12 dS/m), all treatments showed a sharp reduction in callus biomass, with no clear protective effect of plasma.

Influence of Salinity and Cold Plasma on Antioxidant Enzymes (CAT, SOD) and Proline in Callus: Cold plasma treatment and salinity showed significant interactive effects on antioxidant responses in *C. annuum* callus (Figure VI). Catalase (CAT) activity increased with rising NaCl levels, reaching 0.670 U/mL at 12 dS/m in untreated callus, while plasma treatment further enhanced this activity, with the highest value (0.976 U/mL)

recorded at 60 sec under the same salinity level (Figure VIa). Similarly, superoxide dismutase (SOD) activity increased under salinity, peaking at 120.0 U/mL at 10 dS/m in control samples, then declining at 12 dS/m (63.14 U/mL). Plasma treatment significantly enhanced SOD activity, particularly at 180 sec, reaching 145.28 U/mL at 8 dS/m and maintaining higher levels than controls under elevated salinity (Figure VIb), Proline accumulation also increased with both salinity and plasma exposure, with the highest value (41.06 $\mu\text{mol/g}$ FW) observed at 12 dS/m under 180 sec treatment, whereas the lowest level (0.376 $\mu\text{mol/g}$ FW) was recorded in untreated callus under non-saline conditions (Figure VIc).

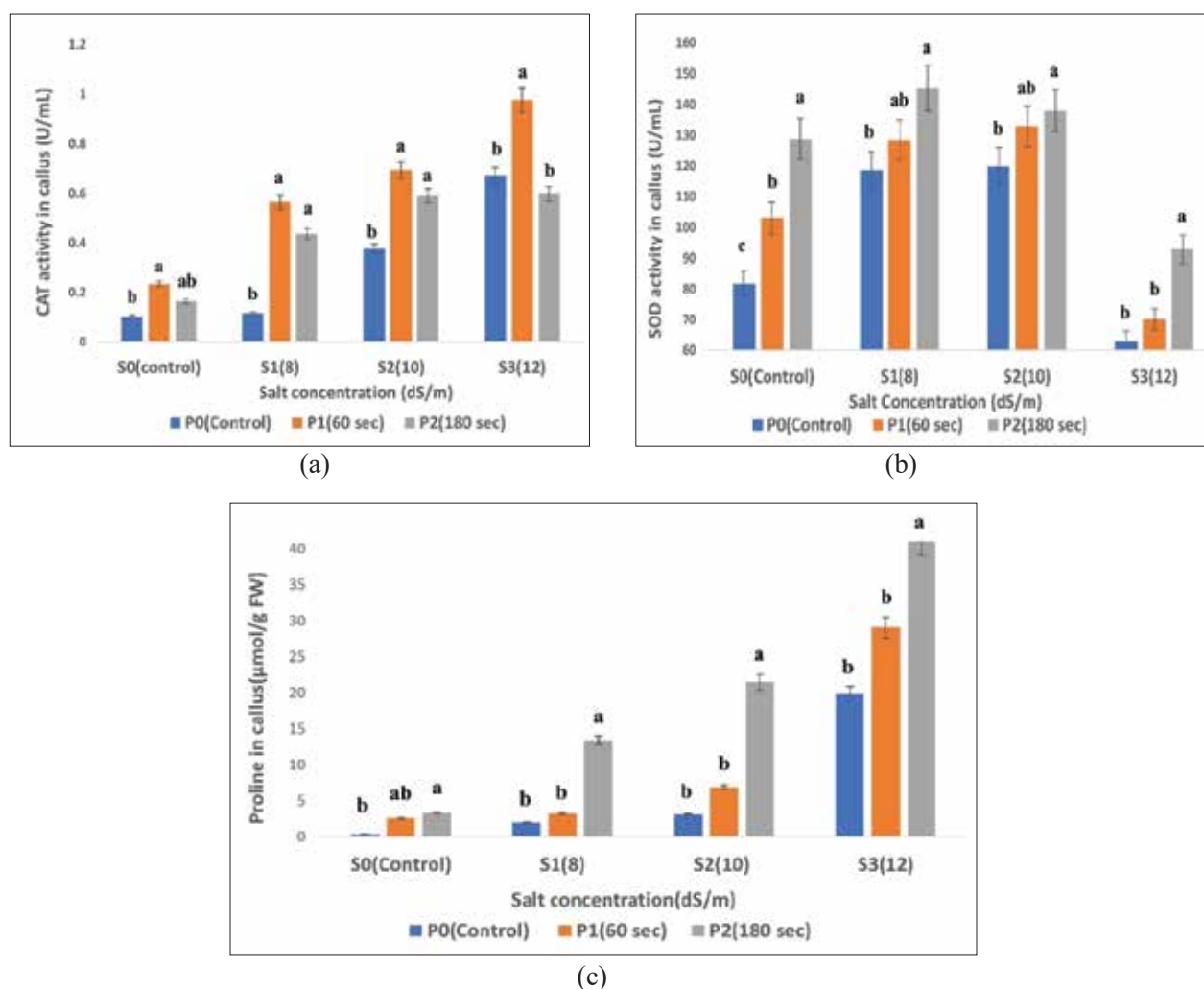


Figure VI: Effect of Different NaCl Concentrations on the Estimation of (a) Catalase Activity (CAT) (U/mL), (b) Superoxide Dismutase Activity (SOD) (U/mL), and (c) the Amino Acid Proline ($\mu\text{mol/g}$ FW), after Cultivating Callus Pieces Treated on MS medium Supplemented with 0.5 NAA mg/L and 1 KIN mg/L for Four Weeks

Synergistic Effect of Cold Plasma and Salinity on CaDHN3 Gene Expression in Sweet Pepper:

Salinity and cold plasma treatments significantly induced CaDHN3 (dehydrin) gene expression in both leaves and callus tissues of *Capsicum annuum* (Figure VIIa). Under salinity stress, expression increased markedly ($P < 0.001$), rising in leaves from 1.00 (control) to 1.97 and 4.55-fold at 8 and 10 dS/m, respectively, while callus showed higher induction (3.12 and 5.77-fold) under the same conditions. Cold plasma further enhanced gene expression, with callus tissues showing the strongest response, reaching 11.22 and 8.67-fold at 60 and 180 sec, respectively, compared to lower increases in leaves (3.03 and 5.56-fold) (Figure

VIIb). Under combined salinity and plasma treatments, *CaDHN3* expression was consistently higher in callus than in leaves. At moderate salinity (8 dS/m) with 60 sec plasma, expression reached 8.00-fold in leaves and 16.91-fold in callus, while at higher salinity (10 dS/m), values increased to 10.85 and 22.47-fold, respectively. Overall, the results demonstrate a clear synergistic effect between cold plasma and salinity stress in upregulating *CaDHN3* expression.

Synergistic Effect of Cold Plasma and Salinity on CaSOS1 Gene Expression in Sweet Pepper:

Expression analysis of the CaSOS1 gene revealed a significant response to salinity, cold plasma, and their interaction (Figure VIII). Under salinity stress,

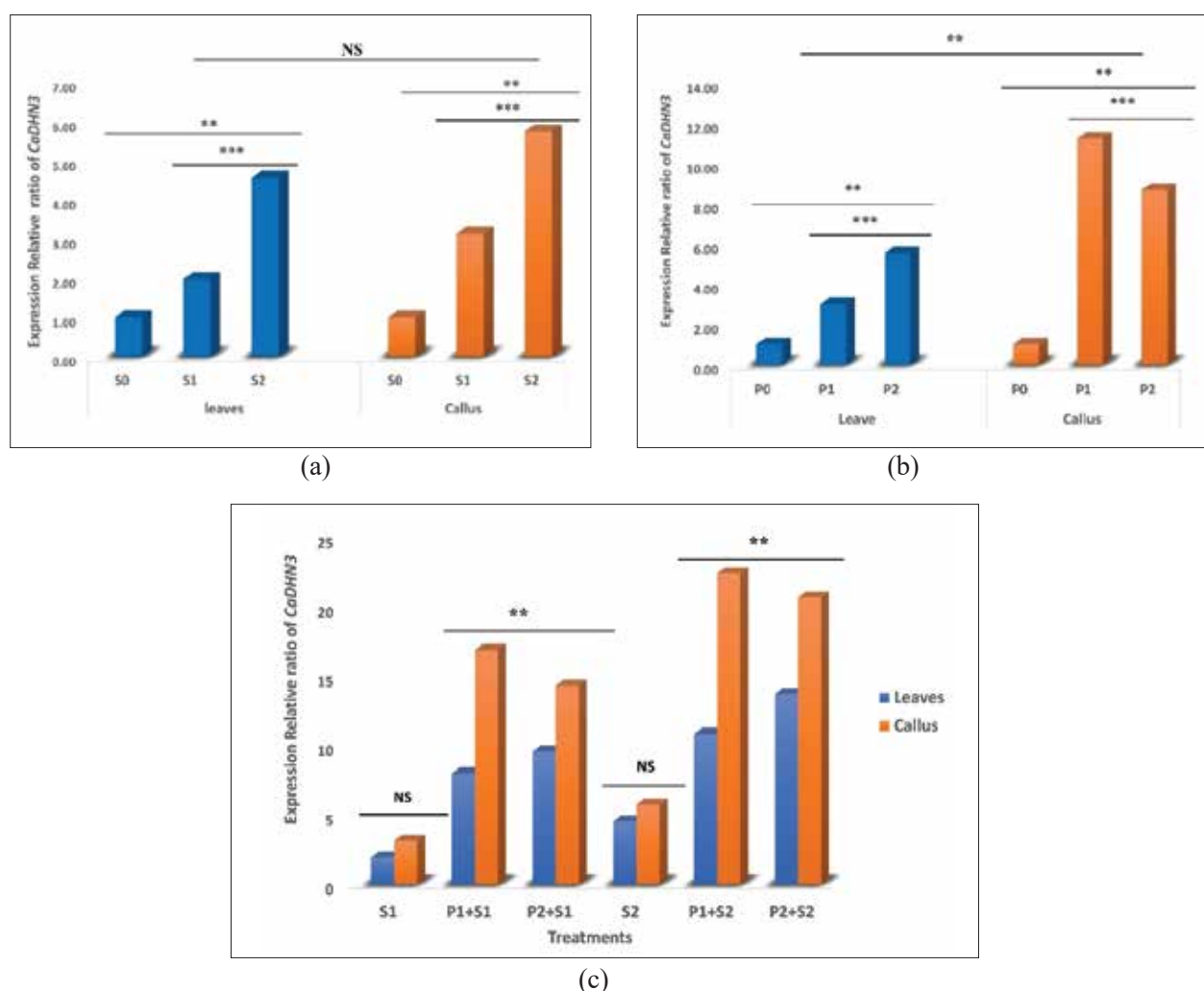


Figure VII: Shows the Gene Expression for CaDHN3 of *Capsicum annuum* due to (a) the Effect of Two Salt Concentrations on Both Leaves and Callus for Pepper, and (b) At Two Time Periods of Plasma on Both Leaves and Callus. (c) The Synergistic Effect of Salt and Plasma at Two Different Concentrations and Durations on Pepper Leaves and Callus

CaSOS1 expression increased markedly ($P < 0.01$), rising in leaves from 1.00 (control) to 5.74-fold at 10 dS/m, while callus showed a stronger response, reaching 7.46-fold under the same conditions (Figure VIIIa). Cold plasma treatment further enhanced gene expression, with the highest induction observed at 180 sec (P2), where expression reached 9.19-fold in leaves and 8.00-fold in callus (Figure VIIIb). Statistical analysis showed a highly significant effect in leaves ($P < 0.001$), whereas callus responses were less variable ($p = 0.420$). Under combined salinity and plasma treatments (Figure VIIIc), expression patterns differed between tissues. Prolonged plasma exposure (180 sec) had a stronger effect in

leaves, whereas callus exhibited greater responsiveness under moderate exposure (60 sec), highlighting tissue-specific regulation of stress responses. Overall, CaSOS1 expression was significantly enhanced by both salinity and plasma treatments, with a clear synergistic effect when combined.

Discussion

Cold plasma (DBD) treatment significantly increased germination speed (GSP) and germination percentage (GP) of sweet pepper seeds compared with the control. The enhancement in germination following plasma treatment may be attributed to increased metabolic and physiological activity, including

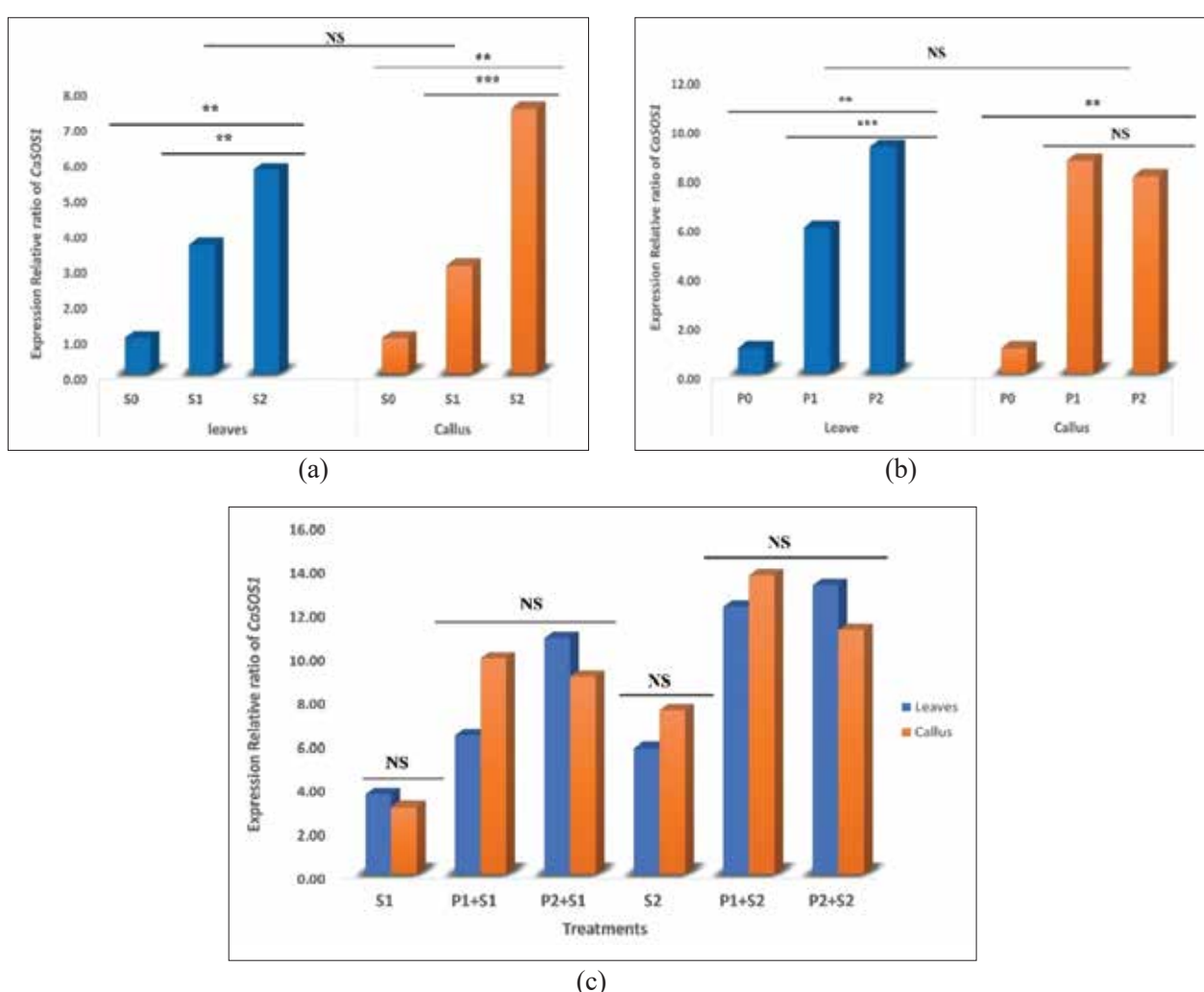


Figure VIII: Shows the Gene Expression for CaSOS1 of *Capsicum annuum* due to (a) The Effect of Two Salt Concentrations on Both Leaves and Callus for Pepper, (b) At Two Time Periods of Plasma on Both Leaves and Callus, and (c) The Synergistic Effect of Salt and Plasma at Two Different Concentrations and Durations on Pepper Leaves and Callus

improved water uptake, enzyme activation, and energy metabolism during early germination. Plasma-generated reactive species may also act as signaling molecules that stimulate germination processes²⁷. Our results agree with previous studies that show plasma treatment can promote plant growth and development and enhance defense mechanisms. Hendeniya et al²⁸ investigated cold atmospheric pressure plasma treatments of the seeds of chili pepper and snake gourd; all treatments (4, 6 & 10 min) significantly enhanced germination and seedling vigor. The decline in germination under salinity stress is mainly due to osmotic imbalance and ionic toxicity, which restrict water absorption and disrupt enzymatic functions. However, plasma treatment alleviated these effects, likely through improving antioxidant defense systems and modifying seed surface properties, thereby reducing oxidative damage. These results are consistent with the previous research^{17,29} which confirmed that cold plasma treatment enhances seed vigor, metabolic activity, and stress tolerance. Cold plasma (DBD) treatment significantly improved the morphological traits of sweet pepper seedlings compared with the control. This enhancement could be associated with the seed vigor and stimulated growth processes in plants, including cell division and elongation, which corresponds with previous studies indicating that plasma treatment suggested beneficial effects for early plant growth^{6,30}. The significant increase in root number suggests improved metabolic activity and root system establishment, which is essential for water uptake³¹ and improving nutrient acquisition efficiency. These mechanisms collectively contribute to maintaining better growth under saline conditions, as supported by previous studies³². Overall, cold plasma treatment, particularly at 180 sec, promoted shoot elongation and root development under both normal and saline conditions.

The enhancement in callus induction and growth following plasma treatment may be attributed to optimized cellular activity under moderate plasma exposure, as observed at 60 sec, whereas prolonged exposure (180 sec) may induce oxidative stress, leading to reduced biomass accumulation³³. This response can be further explained by the interaction between plasma-generated reactive species (ROS and RNS) and plant growth regulators, which promote cellular dedifferentiation and division under balanced auxin–cytokinin conditions³⁴. The decline in callus growth under salinity is mainly due to osmotic stress and ionic toxicity, which inhibit cell division and expansion³⁵. However, plasma treatment improved callus performance under moderate salinity, likely

through enhancing antioxidant activity, maintaining redox balance, and improving nutrient uptake. The absence of a protective effect at 12 dS/m indicates that severe salinity may exceed the tolerance capacity induced by plasma treatment alone. These findings are consistent with previous reports highlighting the role of plasma in enhancing stress tolerance through physiological and biochemical regulation³⁶.

The increase in CAT and SOD activities under salinity reflects the activation of antioxidant defense systems to counteract oxidative stress induced by reactive oxygen species (ROS)^{37–38}. The further enhancement of these enzymes by plasma treatment suggests that plasma priming strengthens ROS detoxification mechanisms, improving cellular protection. The decline in SOD activity at higher salinity (12 dS/m) in control samples may indicate enzyme inhibition under severe stress conditions, whereas plasma-treated calli maintained higher activity, highlighting improved stress tolerance. Proline accumulation under salinity and plasma treatment indicates enhanced osmotic adjustment and cellular protection, as proline acts as an osmoprotectant and stabilizes cellular structures³⁹. The combined increase in enzymatic and non-enzymatic systems suggests that plasma pre-treatment improves redox balance and cellular homeostasis under stress conditions, consistent with previous findings³³.

Salinity and cold plasma treatments significantly affected CaDHN3 gene expression in both leaves and callus tissues of *Capsicum annum*. The significant upregulation of CaDHN3 under salinity stress highlights its essential role in osmotic adjustment, membrane stabilization, and protection against oxidative damage^{8,10}. The higher expression observed in callus tissues indicates a greater sensitivity and responsiveness to stress conditions. The further enhancement of gene expression by cold plasma treatment suggests that plasma-induced reactive oxygen species (ROS) act as signaling molecules that activate stress-responsive pathways⁴⁰. This mechanism likely contributes to improved cellular defense and stress tolerance. The synergistic increase in CaDHN3 expression under combined salinity and plasma treatments, particularly in the callus. These tissue-specific responses may be attributed to the higher metabolic flexibility of callus tissues, as supported by previous studies⁴¹.

The upregulation of CaSOS1 under salinity highlights its key role in Na⁺ transport and ionic homeostasis, reducing sodium toxicity in plant cells⁴². Plasma treatment further enhanced gene expression, likely through ROS-mediated signaling that activates

stress-response pathways. The differential response between leaves and callus indicates tissue-specific regulation, with combined salinity and plasma treatments improving ion balance and cellular stability, particularly alongside CaDHN3 upregulation, thereby enhancing salinity tolerance in sweet pepper.

Conclusion

Cold plasma seed treatment using a dielectric barrier discharge (DBD) system influenced the physiological and molecular responses of *Capsicum annuum* under salinity stress. Plasma treatment improved seed germination, seedling vigor, and shoot and root growth under saline conditions. In addition, plasma exposure increased the activity of antioxidant enzymes, particularly CAT and SOD, and enhanced proline accumulation. At the molecular level, the expression of stress-related genes, including CaDHN3 and CaSOS1, was significantly upregulated, especially in leaf tissues. These responses indicate that cold plasma treatment modulates antioxidant defense and stress-responsive gene expression under salinity conditions. Further studies are required to evaluate the broader physiological responses and potential agricultural applications of this technique.

Acknowledgements

The authors were indebted to all the respondents for their kind cooperation.

Conflict of interest:

The authors would like to state that there is no conflict of interest in this work.

Financial Disclosure

External funding was not obtained for this research.

Authors' contributions

The entire work was done by Sura Muayad Abdul Majeed under the supervision of dr. Mushtak Faraj Karomi Kisko

Data Availability

Any questions regarding the availability of the study's supporting data should be addressed to the corresponding author, who can provide it upon justifiable request.

Ethics Approval and Consent to Participate

The Institutional Review Board granted the study ethical approval. Since this was a cross-sectional study, every study participant provided formal informed consent. Each method followed the appropriate rules and regulations.

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How to cite this article: Majeed SMA, Kisko MFK. Cellular and Molecular Mechanisms of Cold Plasma Action Under Pathogen-Associated Salinity Stress In-Vitro Study Bangladesh J Infect Dis 2026;13(1):113-125

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Article Info

Received on: 10 February 2026

Accepted on: 30 April 2026

Published on: 26 June 2026

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