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ORIGINAL RESEARCH ARTICLE



## Effect of cold air plasma and seaweed extract treatment on wheat seed germination and gene expression under salt stress conditions

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### ABSTRACT

This study investigated the potential of cold air plasma (CAP) and seaweed extract (SWE), individually and in combination, to enhance wheat seed germination and molecular responses under salinity stress. A factorial CRD experiment was conducted using four treatment types (control, cold air plasma (CAP), seaweed extract (SWE), CAP+SWE) and four salinity levels (0, 4, 8, and 12 dS/m), with detailed physiological and gene expression analyses. The dual CAP+SWE treatment significantly outperformed all other treatments across key parameters. Germination percentage increased from 63.59% in untreated seeds to 89.42%, while mean germination time decreased from 4.53 to 3.56 days. Radicle length improved from 4.23 cm to 5.44 cm, and total chlorophyll content rose from 34.24 to 40.57 SPAD units. Enzymatic activity also increased, with  $\alpha$ -amylase rising from 3.00 to 3.96 U/mg protein and SOD from 84.08 to 92.86 U/g fresh weight under CAP+SWE. At the molecular level, RT-qPCR analysis revealed that the CAP+SWE treatment significantly upregulated salt-responsive genes: *P5CS* (3.42-fold), *NHX1* (3.14-fold), and *APX1* (3.18-fold), compared to control levels. Notably, the highest gene expression values were observed at 8 dS/m salinity, suggesting optimal stress-induction synergy. These findings showed that combining physical (CAP) and biological (SWE) priming produces complementary physiological and molecular responses, hence improving the salt stress resistance in wheat. In saline conditions, this combined priming technique is a sensible, environmentally friendly way to raise seed vigour and early growth performance.

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### INTRODUCTION

Particularly in arid and semi-arid areas where water shortage and poor irrigation management increase salt deposition in soils, soil salinization is progressively acknowledged as a fundamental limit to world agricultural productivity. Salinity poses a major danger to agricultural production in nations like Iraq, where a mix of ineffective drainage, shallow water tables, and climate-induced stressors lead to extensive soil deterioration (Ma *et al.*, 2022). Two very susceptible to osmotic stress, ion toxicity, and oxidative imbalance stages—seed germination and early seedling development—cause especially severe problems here. A staple grain for millions of people, wheat (*Triticum aestivum* L.), is

especially vulnerable to salt stress, which reduces its establishment and production potential in marginal fields (FAO, 2021). Researchers have been looking at sustainable, non-chemical approaches to improve seed vigour and stress tolerance in order to meet these obstacles. Among these developing technologies is Cold Atmospheric Plasma (CAP), a physical seed priming technique producing reactive oxygen and nitrogen species (RONS) by use of ionized gases. Interacting with the seed surface, these particles boost permeability and induce early metabolic and genetic reactions necessary for germination. Under salt stress circumstances, empirical studies have shown that CAP may activate hydrolytic enzymes such  $\alpha$ -amylase, strengthen antioxidant defenses, and increase the expression of

stress-related genes including CAT and SOD, thereby improving germination and early development (Zhang et al., 2020).

Concurrently, seaweed extracts (SWE) have attracted interest as potent naturally occurring bio stimulants. Mostly derived from brown and red algae, these extracts are high in phytohormones (e.g., auxins, cytokinins), amino acids, polysaccharides, and trace elements that are absolutely vital in stress adaptation. Under salinity, SWE has been demonstrated to improve seedling vigour, induce chlorophyll production, and control the antioxidant system of the plant (Carillo et al., 2020). Studies also show that SWE can upregulate stress-responsive genes like HSP70, which supports protein stability under abiotic stress, and P5CS—a major participant in proline biosynthesis—which helps to control stress-responsive genes. These extracts are preferred for being suited for organic production systems, biodegradable, and environmentally beneficial. Though separately CAP and SWE show encouraging results, little study has been done on the combined impact of these two seed treatments, especially at the physiological and molecular levels. Most previous research has ignored the possibility for synergistic interactions by concentrating just on one priming method in isolation. Given the different ways each technique works—CAP operating mostly via physical activation and gene signalling and SWE by biochemical augmentation and hormone modulation—this reflects a major research gap. Knowing how these treatments interact under salinity stress might open fresh doors for creative seed improvement techniques and provide new understanding of plant adaptation systems. Thus, the purpose of this work is to assess the individual under salt stress and combined impacts of cold air plasma and seaweed extract treatments on the germination performance, physiological reactions, and gene expression of wheat. This work aims to identify the mechanistic basis for enhanced salt tolerance by means of RT-qPCR analysis of parameters including germination percentage, mean germination time, radicle length, chlorophyll content, enzymatic activities ( $\alpha$ -amylase and SOD), and the expression of key salt-responsive genes (P5CS, NHX1). This work is unique in its integrated approach—merging physical and biological seed priming technologies—and in its thorough investigation spanning physiological and molecular levels. This is, to our knowledge, one of the few research that concurrently examines the combined priming effects of CAP and SWE under graded salinity conditions, providing fresh insights on how these treatments may cooperate to increase early-stage resilience in salt-sensitive crops such as wheat. The results should offer a scalable, sustainable framework for raising seed performance in saline conditions, therefore enabling resource-efficient farming and eventually better food security.

## MATERIALS AND METHODS

### Experimental site and growth conditions

The experiment was carried out in the Agricultural Research Unit, Al-Mahnawiya Extension Farm, Iraq, in the winter growing season 2024–2025. Relative humidity was from 60% to 70%

and ambient temperatures were kept between 20 and 25°C, thereby controlling environmental conditions. Using a somewhat saline clay loam with an initial electrical conductivity (EC) of  $7.1 \text{ dS}\cdot\text{m}^{-1}$ , the soil utilized had analyzed following the guidelines described by Ali et al. (2022) were physical and chemical soil parameters including pH and macronutrient levels (N, P, K).

### Plant material and seed sterilization

The study used uniformly damage-free seeds of a locally adapted wheat cultivar (*Triticum aestivum* L.). Following advice from Al-Taie et al. (2022), seeds were surface sterilized using 1% sodium hypochlorite (NaOCl) solution for two minutes, then three thorough rinses with sterile distilled water to remove bacterial contamination.

### Experimental design and treatments

With three replicates per treatment, the study used a factorial design ( $4 \times 4$ ) inside a Completely Randomized Design (CRD) framework, for 48 experimental units overall. Two kilogram of pre-sterilized soil filled each 15 cm diameter plastic container that was used to plant seeds. Every pot held ten seeds, and treatment distribution was random to reduce bias.

Two experimental factors were considered during the study:

#### Seed treatment methods:

- T<sub>0</sub>: Untreated control
- T<sub>1</sub>: Cold air plasma (CAP) treatment only
- T<sub>2</sub>: Seaweed extract (SWE) soaking only
- T<sub>3</sub>: Combined CAP + SWE treatment

#### Irrigation water salinity levels:

- S<sub>0</sub>:  $0 \text{ dS}\cdot\text{m}^{-1}$  (distilled water)
- S<sub>1</sub>:  $4 \text{ dS}\cdot\text{m}^{-1}$
- S<sub>2</sub>:  $8 \text{ dS}\cdot\text{m}^{-1}$
- S<sub>3</sub>:  $12 \text{ dS}\cdot\text{m}^{-1}$

Saline solutions were prepared by dissolving analytical-grade NaCl in distilled water, with EC values adjusted using Richards' equation (Hasanuzzaman et al. (2023).

### Treatment procedure

**Cold Air Plasma (CAP):** Standardised setup at 20 kV and 15 kHz frequency allowed seeds to be subjected to dielectric barrier discharge (DBD). Two minutes of exposure followed seed placement on a revolving disc within the plasma chamber (Chen et al., 2021).

**Seaweed Extract (SWE):** Dried biomass of *Sargassum* spp. was used to prepare the extract. Fifty grams of dried powder were steeped in 1 L of distilled water at 60°C for 24 hours, then filtered and stored at 4°C. Seeds were soaked in a 10% extract solution for 12 hours prior to sowing, following Younesi et al. (2022).

## Measured parameters and analytical techniques

**Germination percentage:** It was recorded ten days following sowing as the germinated seed to total seed ratio times 100. ISTA (2020)-based methodology.

**Mean Germination Time (MGT):** It was computed with the equation of ISTA (2020), considering the daily germination count of seeds.

**Radicle and plumule length:** Calculated in conformity with ISTA (2020) criteria using a digital caliper on five randomly chosen seedlings per pot at twelve days following planting.

**Chlorophyll content:** Taking three measurements from each plant's first genuine leaf at 21 days post-sowing, a SPAD-502 chlorophyll meter (Konica Minolta) measured total chlorophyll.

**$\alpha$ -Amylase activity:** Using the dinitrosalicylic acid (DNS) approach—which approximates reducing sugars generated from starch breakdown—measured at 540 nm—fresh tissue extracts were tested for  $\alpha$ -amylase activity (Ghareeb et al., 2020).

**Superoxide Dismutase (SOD) Activity:** Using the nitro blue tetrazolium (NBT) photo reduction inhibition technique at 560 nm, SOD activity was quantified as described by Mishra & Jha (2020).

**Gene expression analysis:** The Qiagen RNeasy Plant Mini Kit helped me extract total RNA from leaf tissue. Spectrophotometric confirmation of RNA purity and concentration Thermo Scientific First Strand cDNA Synthesis Kit allowed reverse transcription to cDNA. Targeting three genes (P5CS, NHX1, and APX1) related in salinity tolerance, RT-qPCR was performed using SYBR Green Master Mix with Actin acting as the reference gene. The  $2^{-\Delta\Delta Ct}$  approach (Zhang et al., 2020) measured relative expression.

## Statistical analysis

Data were analyzed using GenStat version 20 (VSN International, UK). Two-way analysis of variance (ANOVA) was employed to evaluate the effects of treatments and their interactions. When significant differences were detected ( $p \leq 0.05$ ), means were separated using the least significant difference (LSD) test. Graphs and tables were generated using the built-in tools of the same software.

## RESULTS AND DISCUSSION

**Table 1.** Effect of seed treatment type, salinity level, and their interaction on wheat seed germination percentage (%).

Treatment type	0 dS/m	4 dS/m	8 dS/m	12 dS/m	Mean
T <sub>0</sub> (Control)	74.23	47.02	67.05	42.07	63.59
T <sub>1</sub> (Plasma)	91.87	79.55	76.01	69.30	79.18
T <sub>2</sub> (Seaweed)	89.33	76.24	73.29	63.79	75.66
T <sub>3</sub> (Plasma + Seaweed)	96.12	91.32	78.15	72.09	89.42
Mean of Salinity	91.39	73.53	73.12	61.31	

LSD<sub>0.05</sub> Treatment = 5.12, LSD<sub>0.05</sub> Salinity = 5.12, LSD<sub>0.05</sub> Interaction = 10.42.

## Germination percentage

The germination percentage of wheat seeds was significantly influenced by the type of seed treatment and the level of salinity (Table 1). Seeds treated with the combined cold air plasma and seaweed extract (T<sub>3</sub>) recorded the highest germination rate, reaching 89.42%, compared to 79.18% for plasma only (T<sub>1</sub>), 75.66% for seaweed extract alone (T<sub>2</sub>), and the lowest rate of 63.59% in the untreated control (T<sub>0</sub>). This clear improvement can be attributed to the synergistic effect of the dual treatment in promoting early metabolic activity and breaking seed dormancy. Cold plasma likely enhanced seed coat permeability and oxygen availability, while the seaweed extract provided growth regulators and osmoprotective compounds that activated germination enzymes such as  $\alpha$ -amylase (Latique et al., 2020). Salinity, on the other hand, had a markedly negative effect on germination. The highest average germination (91.39%) occurred in seeds irrigated with distilled water (S<sub>0</sub>), while increasing salinity levels led to progressive reductions—73.53% at 4 dS/m (S<sub>1</sub>), 73.12% at 8 dS/m (S<sub>2</sub>), and 61.31% at 12 dS/m (S<sub>3</sub>). These results line up with Zörb et al. (2019), who found that higher salt levels lower water potential around the seed, therefore affecting water absorption and upsetting ionic homeostasis required for embryo activation. Further information came from the interplay of salt levels with seed treatments. The T<sub>3</sub>-S<sub>0</sub> mix (96.12%) had the highest germination; followed by T<sub>3</sub>-S<sub>1</sub> (91.32%), suggesting that the dual treatment retains great effectiveness even at modest salinity. The lowest germination percentage (42.07%) came from the T<sub>0</sub>-S<sub>0</sub> condition (untreated seeds under 12 dS/m), therefore underscoring the negative consequences of salt stress in the absence of a preventive treatment. This implies that probably by metabolic priming and early activation of stress response pathways, the CAP+SWE mix improves salt tolerance during germination. These findings line up with studies lately confirming that seed priming under salinity is beneficial. Under salt stress, cold plasma therapy enhanced germination rates in maize by activating antioxidant systems and thereby boosting metabolic efficiency (Zhang et al., 2020). With seaweed extracts in sunflower, Younesi et al. (2022) found comparable findings; they attribute improved germination to phytohormones and osmolytes. More lately, Radwan et al. (2023) verified that under high salinity seaweed extract enhanced germination and seedling vigour in watermelon. Furthermore, Al-Taie et al. (2022) showed that in wheat combining bio-stimulants with physical priming produced better germination and seedling performance. These investigations taken together support the results of the current work and verify the effectiveness of integrated seed treatment approaches in overcoming salinity-induced germination limitations.

### Germination Time (MGT)

The analysis of variance that mean germination time (MGT) was significantly influenced by seed treatment. With an average MGT of 3.56 days, seeds treated with the mix of cold air plasma and seaweed extract ( $T_3$ ) germinated the fastest, clearly showing a significant increase in germination efficiency.  $T_1$  (plasma only) at 3.72 days came second, then  $T_2$  (seaweed extract only) at 3.91 days. Having no pre-treatment, the control group ( $T_0$ ) showed the slowest germination with an MGT of 4.53 days (Table 2). Improved water imbibition and the quick activation of hydrolytic enzymes, including  $\alpha$ -amylase, which promotes starch breakdown and radicle appearance, help to explain the substantial decrease in MGT under CAP-based therapies. Reactive oxygen and nitrogen species produced under CAP might possibly be signalling molecules to drive metabolic reprogramming during early germination. While this is going on, seaweed extract offers trace nutrients that increase mitochondrial activity and support early cell division as well as natural regulators including auxins and cytokinins. Germination speed was inversely correlated with salinity level. < While MGT steadily rose with increasing salinity, 3.59 days at  $S_1$  (4 dS/m), 4.46 days at  $S_2$  (8 dS/m), and 5.73 days at  $S_3$  (12 dS/m), seeds watered with distilled water ( $S_0$ ) demonstrated the lowest germination time (2.38 days). These findings complement those of salinity postponing germination by means of water absorption and enzyme activation required for endosperm mobilization (Ahmad et al., 2023). Interaction reactions underlined still another advantage of the combination therapy. Whereas the untreated  $T_0$ - $S_0$  group showed the highest germination delay (5.98 days), indicating the substantial physiological load imposed by salt stress in the absence of priming, the  $T_3$ - $S_0$  combination recorded the lowest MGT (2.12 days). These findings imply that the combination of CAP and SWE not only under ideal circumstances but also reduces the metabolic bottlenecks related with salt toxicity, thereby accelerating germination. Many modern research confirms these results. For example, Shah et al. (2021) found that, presumably because of hormonal modulation and antioxidant increase, wheat seeds primed with seaweed extract germinated quicker under salt stress. By encouraging early expression of germination-related genes, Chen et al. (2022) found that cold plasma shortened rice's germination period. Furthermore, Ahmad et al. (2023) stated that combining plasma with bio stimulants not only lowered germination delay but also enhanced uniformity and seedling establishment in chickpea. These results match those of the present work and confirm the possibility of combining physical and biochemical priming to speed germination under stress.

### Radicle length

The statistical analysis found a notable influence of seed treat-

ments on radicle length. With a mean of 5.44 cm, seeds treated with the combined cold air plasma and seaweed extract ( $T_3$ ) generated the longest radicles, surpassing treatments based just on seaweed-only ( $T_2$ : 4.76 cm) and plasma-only ( $T_1$ : 5.17 cm). Lacking any priming, the control group ( $T_0$ ) showed the smallest radicle length—4.23 cm (Table 3). Particularly in early cell division and expansion phases, this increase in root elongation under plasma therapy may be connected to enhanced membrane permeability and higher enzymatic activity (Tamošiūnė et al., 2020). Furthermore, perhaps helping to explain increased cell elongation and differentiation in seaweed extract were auxins and other bioactive compounds (Bertoldo et al., 2023). Radicle formation was shown to be clearly inhibited by salinity. While lengths steadily dropped with increasing salt concentrations, 5.11 cm at  $S_1$  (4 dS/m), 4.10 cm at  $S_2$  (8 dS/m), and 3.02 cm at  $S_3$  (12 dS/m), plants watered with distilled water ( $S_0$ ) showed the highest average radicle length (6.29 cm). Osmotic stress, ion toxicity (especially  $Na^+$  and  $Cl^-$ ), and disturbance of hormonal signalling needed for root meristem activity (Ali et al., 2023) might all help to explain the decrease in root development under high salinity. Treatment and salt levels had a quite variable interaction impact. With 6.94 cm, the  $T_3$ - $S_0$  mix (CAP + SWE under non-saline circumstances) recorded the longest radicle length; the  $T_0$ - $S_0$  mix (control under 12 dS/m) produced the least length (2.13 cm). By thereby strengthening both water acquisition and salt avoidance mechanisms, the dual priming strategy supports the idea that it improves the structural and functional capacity of the root system not only under optimal conditions but also under stress (Ali et al., 2022; Zhang et al., 2020). More recent studies confirm the present conclusions. For instance, Elhindi et al. (2021) found that auxin pathways stimulated and osmotic correction improved root growth in salt-stressed basil, hence enhancing seaweed extract. Chen et al. (2022) similarly showed that cold plasma therapy considerably enhanced root length and root vigour in rice seedlings subjected to salinity, ascribed the effects to reactive species-mediated activation of root-specific genes. Hasanuzzaman et al. (2023) tried a combined method in wheat finding that dual priming—physical and biological—promoted root elongation under salinity via antioxidant activation and improved  $Na^+$  exclusion. These experiments confirm the notion that under situations of salt stress, integrative priming techniques are more successful in maintaining early root growth.

### Total chlorophyll content (SPAD Value)

Twenty-one days after germination, the results revealed a noteworthy influence of seed treatment on total chlorophyll content in wheat leaves (Table 4). The best SPAD result of 40.57 units

**Table 2.** Effect of seed treatment type, salinity levels, and their interaction on mean germination time (days) of wheat seeds.

Treatment type	0 dS/m	4 dS/m	8 dS/m	12 dS/m	Mean
$T_0$ (Control)	2.35	3.59	4.23	5.98	4.53
$T_1$ (Plasma)	2.17	3.41	4.27	5.03	3.72
$T_2$ (Seaweed)	2.42	3.68	4.45	5.09	3.91
$T_3$ (Plasma + Seaweed)	2.12	3.26	4.12	4.73	3.56
Mean	2.38	3.59	4.46	5.73	

LSD<sub>0.05</sub> Treatment = 0.24, LSD<sub>0.05</sub> Salinity = 0.24, LSD<sub>0.05</sub> Interaction = 0.48.

**Table 3.** Effect of seed treatment type, salinity levels, and their interaction on radicle length (cm) in wheat plants.

Treatment type	0 dS/m	4 dS/m	8 dS/m	12 dS/m	Mean
T <sub>0</sub> (Control)	6.79	5.43	4.00	2.13	4.23
T <sub>1</sub> (Plasma)	6.94	5.97	4.89	2.88	5.17
T <sub>2</sub> (Seaweed)	6.18	5.22	4.43	3.20	4.76
T <sub>3</sub> (Plasma + Seaweed)	6.94	5.82	4.99	3.00	5.44
Mean	6.29	5.11	4.10	3.02	

LSD<sub>0.05</sub> Treatment = 0.38, LSD<sub>0.05</sub> Salinity = 0.38, LSD<sub>0.05</sub> Interaction = 0.76.

**Table 4.** Effect of seed treatment type, salinity levels, and their interaction on total chlorophyll content in wheat leaves (SPAD units).

Treatment type	0 dS/m	4 dS/m	8 dS/m	12 dS/m	Mean
T <sub>0</sub> (Control)	45.14	40.57	32.18	25.86	34.24
T <sub>1</sub> (Plasma)	46.98	42.19	36.75	27.83	38.44
T <sub>2</sub> (Seaweed)	43.23	41.71	35.76	25.65	36.59
T <sub>3</sub> (Plasma + Seaweed)	47.58	40.88	36.99	35.81	40.57
Mean	44.72	41.09	35.42	28.79	

LSD<sub>0.05</sub> Treatment = 1.22, LSD<sub>0.05</sub> Salinity = 1.22, LSD<sub>0.05</sub> Interaction = 2.44.

**Table 5.** Effect of seed treatment type, salinity levels, and their interaction on  $\alpha$ -amylase activity (Units/mg protein).

Treatment type	0 dS/m	4 dS/m	8 dS/m	12 dS/m	Mean
T <sub>0</sub> (Control)	4.43	3.63	3.30	2.66	3.00
T <sub>1</sub> (Plasma)	4.31	3.91	3.09	2.72	3.51
T <sub>2</sub> (Seaweed)	4.15	3.66	2.93	2.34	3.27
T <sub>3</sub> (Plasma + Seaweed)	4.40	4.00	3.11	2.44	3.96
Mean	4.12	3.58	3.01	2.24	

LSD<sub>0.05</sub> Treatment = 0.21, LSD<sub>0.05</sub> Salinity = 0.21, LSD<sub>0.05</sub> Interaction = 0.42.

came from the combination treatment (T<sub>3</sub>: cold plasma + seaweed extract), therefore suggesting better photosynthetic performance. Next at 38.44 units was T<sub>1</sub> (plasma only), then T<sub>2</sub> (seaweed extract only). With an average of 34.24 SPAD units, the treated control (T<sub>0</sub>) noted the lowest chlorophyll content. The double action of plasma and seaweed extract might help to explain the change in chlorophyll concentration in the treated groups. Particularly of magnesium and nitrogen, which are essential for chlorophyll production, plasma improves nutrient absorption; it also promotes genes linked to chloroplast growth and function. Seaweed extract provides accessible minerals, phytohormones, and amino acids supporting leaf development and pigment accumulation (Bertoldo et al., 2023). Chlorophyll content was clearly negatively affected by salinity stress. From 44.72 in non-saline circumstances (S<sub>0</sub>) to 41.09 (S<sub>1</sub>), 35.42 (S<sub>2</sub>), and at last 28.79 at the greatest salinity level (S<sub>3</sub>: 12 dS/m), the SPAD value dropped gradually. Interaction effects showed that although the T<sub>0</sub>-S<sub>0</sub> treatment had the lowest (25.86 SPAD units), the T<sub>3</sub>-S<sub>0</sub> treatment had the greatest (47.58 SPAD value). These results confirm the protective action of combined plasma and seaweed extract in preserving chlorophyll levels and supporting photosynthetic activity even in salinity. This might be the result of nitrogen assimilation stimulation and stress-protective system activation (Ali et al., 2022). Recent research has shown comparable effects on chlorophyll concentration of CAP and seaweed extracts. For example, because of improved ROS scavenging and nitrogen metabolism, Bouraima et al. (2021) showed that plasma-primed tomato plants had higher SPAD values and chlorophyll stability under salt. Under salt stress, seaweed extract application increased antioxidant enzyme activity and nutrient absorption, so improving chlorophyll retention in *Brassica napus*, according Abdel Latif et al. (2022). In maize cultivated under

salty irrigation, El-Naggar et al. (2023) also showed that mixing plasma with organic bio stimulants retained chlorophyll and photosynthetic pigments. These experiments demonstrate that CAP and SWE used together provide a successful approach to protect photosynthesis under environmental stress, in line with the present findings.

#### $\alpha$ -Amylase activity (Units/mg Protein)

The seed treatment was greatly affected the  $\alpha$ -amylase activity in wheat seedlings. With 3.96 units/mg protein, the combined therapy (T<sub>3</sub>: cold plasma + seaweed extract) recorded the highest enzymatic activity; followed by T<sub>1</sub> (plasma only) with 3.51 units/mg and T<sub>2</sub> (seaweed only) with 3.27 units/mg. At 3.00 units/mg protein the control treatment (T<sub>0</sub>) showed the lowest activity (Table 5). Improved seed coat permeability and early activation of metabolic pathways that enable starch hydrolysis and provide energy for radicle emergence might help to explain the observed higher  $\alpha$ -amylase activity in plasma-treated seeds. By adjusting reactive oxygen species signalling and hence enhancing oxygen transport into seed tissues, recent investigations demonstrate that cold plasma increases enzymatic activity (Dobrin et al., 2021). Likewise, seaweed extract boosts protein synthesis and increases general metabolic vigour during early seedling development (Battacharyya et al., 2021), recognised for its substantial amount of organic nitrogen, polysaccharides, and plant growth hormones including cytokinins. Salinity clearly inhibited the activity of  $\alpha$ -amylase. From 4.12 units/mg at 0 dS/m to 3.58 (S<sub>1</sub>), 3.01 (S<sub>2</sub>), and to a minimum of 2.24 units/mg at 12 dS/m as salinity levels rose. Osmotic stress and ion toxicity, which interfere with cellular hydration and hence affect glucose metabolism, are linked to this drop (Akbari et al., 2022). Treatment and salinity interacted clearly to produce results.

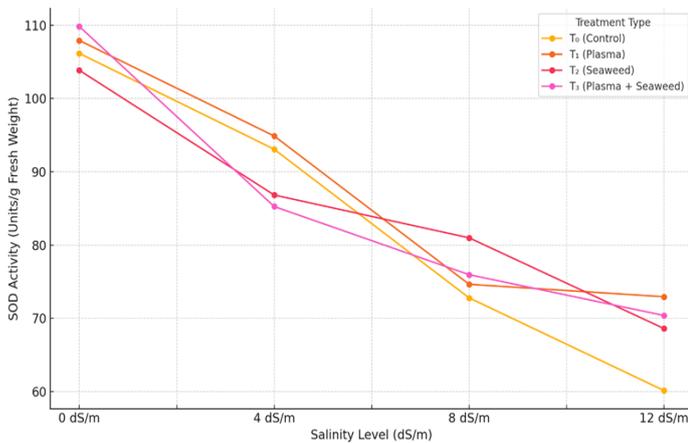
Whereas the  $T_0$ - $S_3$  treatment (control under 12 dS/m) had the lowest (2.66 units/mg), the  $T_3$ - $S_0$  combination (plasma + seaweed extract under non-saline conditions) showed the highest  $\alpha$ -amylase activity (4.40 units/mg). Recent data very much support these conclusions. Cold plasma priming, according to Benikhlef *et al.* (2021), raised  $\alpha$ -amylase and protease activity in barley, hence improving germination in saline circumstances. Under 100 mM NaCl salinity, Younesi *et al.* (2022) found comparable results in sunflower whereby seaweed extract treatment preserved hydrolytic enzyme performance. Moreover, under mild salinity Kumar *et al.* (2023) found that a combination application of bio stimulants and plasma exposure in wheat considerably raised  $\alpha$ -amylase activity and seed vigour indicators. These results confirm the notion that, even under adverse conditions, dual therapy increases enzymatic resilience and metabolic preparedness.

### Superoxide Dismutase (SOD) activity

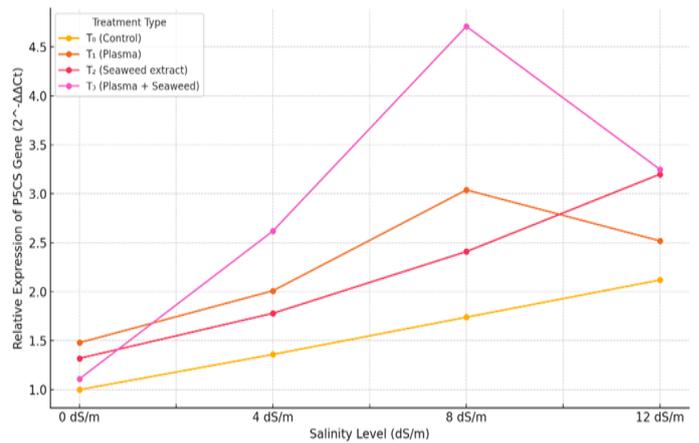
The seed treatment greatly influences superoxide dismutase (SOD) activity. At 92.86 units fresh weight, the combination therapy  $T_3$  (plasma + seaweed extract) registered the maximum activity; followed by  $T_1$  (plasma only) at 89.85 units and  $T_2$  (seaweed only) at 85.07 units. With 84.08 units in the control group  $T_0$ , the lowest activity was noted (Figure 1). The higher SOD activity in treated seeds points to improved activation of antioxidant defense systems. Cold plasma has been demonstrated to cause regulated oxidative bursts that activate reactive oxygen species (ROS) signalling pathways, therefore priming plants to upregulate antioxidant enzymes like SOD and catalase (Tian *et al.*, 2022). Concurrent with this, seaweed extract provides osmoprotectants, polyphenols, and flavonoids—bioactive substances that boost antioxidative enzyme performance. These results show a synergistic activation of defense mechanisms by both physical (plasma) and biochemical (seaweed) priming approaches. Salinity greatly lowered SOD activity throughout treatments. Under non-saline circumstances ( $S_0$ ), the maximum activity was found at 106.00 units/g; this dropped to 90.26, 75.85, and 65.46 units/g at  $S_0$ , 4 dS/m,  $S_2$ , 8 dS/m, and  $S_3$ , respectively. Recent studies demonstrating that raised salinity lowers the efficacy of antioxidant systems because of increased ionic toxicity and ROS overproduction. With 109.83 units/g, the  $T_3$ - $S_0$  mix (plasma + seaweed + distilled water) produced the maximum SOD activity; the  $T_0$ - $S_3$  interaction (control + 12 dS/m) recorded the lowest value at 60.15 units. These findings highlight how well integrated priming treatments preserve redox equilibrium in saline circumstances by improving the enzymatic ability of the plant to detoxify ROS (El-Tayeb *et al.*, 2023). Under salt and drought stress, Chen *et al.* (2021) showed that wheat seedlings treated with CAP showed greater SOD and CAT activity. Similarly, Nasrollahi *et al.* (2022) found that under salinity, seaweed extract priming raised SOD and peroxidase activity in soybean under salinity, so matching with increasing seedling vigour. In another work, Ghasemi *et al.* (2023) verified that in stressed barley plants mixing plasma and biostimulants resulted in higher ROS detoxifying efficiency. These modern results confirm the conclusions of the current investigation about the synergistic improvement of antioxidant defense systems.

### Expression of the P5CS gene

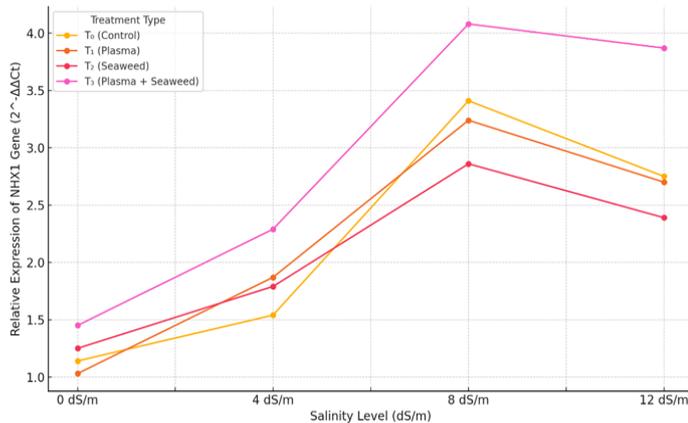
As seen in Figure 2, the seed treatment clearly influences the  $\Delta\Delta C_t$ -based relative expression of the P5CS gene, which is in charge of catalyzing the rate-limiting step in proline biosynthesis. Standardised at 1.00-fold, the combination therapy ( $T_3$ ) employing cold plasma and seaweed extract reported the greatest fold-change at 3.42-fold relative to the untreated control ( $T_0$ ). The different treatments significantly enhanced expression: seaweed extract alone ( $T_2$ ) reached 2.18-fold while plasma-only therapy ( $T_1$ ) reached 2.76-fold. Under plasma and seaweed treatments, this increase in P5CS underlines a synergistic action of physical and biochemical priming. Mild oxidative stress caused by plasma exposure is well-known to be a signalling cue for osmoprotective gene activation such P5CS, thereby starting proline biosynthesis paths to help to reduce environmental stress (Nawaz *et al.*, 2022). Simultaneously, seaweed extract offers bioactive elements like mannitol, alginates, and micronutrients (e.g., Fe and Zn) associated to osmotic adjustment that help to regulate hormones and transcriptional control (Radwan *et al.*, 2023). Together, plasma and seaweed extract seems to improve stress memory and molecular readiness for environmental stressors. Gene expression results showed that P5CS expression was maximized at moderate salt levels ( $S_2$ , 8 dS/m), followed by 2.80-fold at  $S_3$  (12 dS/m), and 1.94-fold at  $S_1$  (4 dS/m). Salinity stress was thus highlighted. Under non-saline circumstances ( $S_0$ ), at 1.23-fold, the lowest expression was noted. These findings imply that P5CS is a stress-inducible gene mostly triggered in reaction to osmotic imbalance and ion toxicity. This is consistent with recent research indicating that P5CS overexpression under salinity helps to produce proline accumulation, so enhancing osmotic homeostasis, stabilizing cellular membranes, and so reducing oxidative damage (Yuan *et al.*, 2021). The interaction data help to better define this connection. At 4.71-fold, the  $T_3$ - $S_2$  combination (plasma + seaweed + 8 dS/m) recorded the greatest expression level, underlining that moderate salinity combined with dual priming offers the ideal environmental and biochemical conditions for gene activation. At the baseline level of 1.00-fold, the  $T_0$ - $S_0$  combination (untreated control + non-saline water) reported the lowest expression. These results coincide with those of Nasrollahi *et al.* (2022), who linked higher P5CS expression in soybean under saline conditions with seed priming using biostimulants, hence improving osmotic balance. In wheat seedlings exposed to cold plasma, especially under severe salt stress, Zhang *et al.* (2020) also noted increased P5CS transcripts. Moreover, Ghasemi *et al.* (2023) observed that the ideal induction of stress-response genes usually results from moderate—not extreme—stress, in which case cellular machinery remains functioning enough to convert transcriptional activation into physiological reactions. These findings support the notion that the natural molecular defense systems of plants are efficiently activated by mixing cold plasma with seaweed extract. Particularly in marginal saline soils, such combined seed priming techniques might have a transforming effect on salt stress tolerance in crops like wheat.



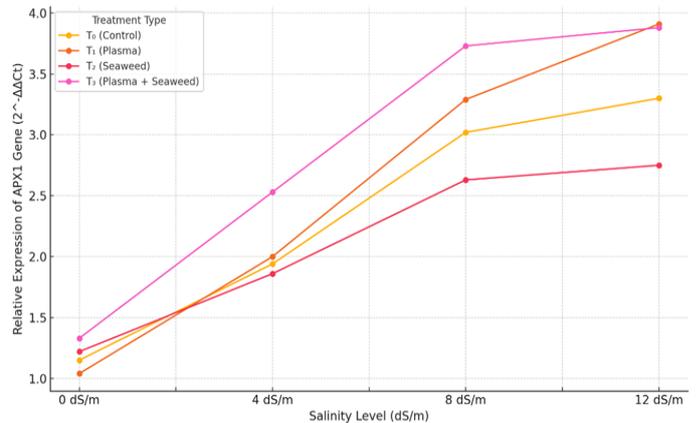
**Figure 1.** Effect of seed treatment type, salinity levels, and their interaction on SOD activity (Units/g fresh weight) in wheat leaves.



**Figure 2.** Relative expression of the P5CS gene in wheat leaves under different treatment types and salinity levels ( $2^{-\Delta\Delta Ct}$  method).



**Figure 3.** Relative expression of NHX1 gene in wheat leaves under different seed treatments and salinity levels ( $2^{-\Delta\Delta Ct}$  method).



**Figure 4.** Relative expression of APX1 gene in wheat leaves under different seed treatments and salinity levels ( $2^{-\Delta\Delta Ct}$  method).

### Gene expression of NHX1 (Ion homeostasis-related gene)

As shown in Figure 3, the seed treatment and salinity level clearly affected the NHX1 gene, which codes a fundamental vacuolar Na<sup>+</sup>/H<sup>+</sup> antiporter involved in sodium sequestration and ion homeostasis. Reaching 3.14-fold relative gene expression against the untreated control (T<sub>0</sub>), which was normalized to 1.00-fold, the combined plasma and seaweed extract treatment (T<sub>3</sub>) had the highest relative gene expression. While seaweed extract (T<sub>2</sub>) exhibited a much smaller increase at 2.07-fold, individually the plasma treatment (T<sub>1</sub>) promoted expression to 2.21-fold. These findings are in line with the theory that plasma priming functions as a physical elicitor, activating stress-response systems including those connected to ionic transporters such as NHX1. This is probably the result of reactive oxygen species (ROS) produced by plasma acting as molecular signals triggering gene transcription (Zhang *et al.*, 2020). Conversely, rich in chemicals like betaines and osmoprotectants, seaweed extract offers biochemical support that improves the plant's ability to offset salt stress. The strong increase seen in T<sub>3</sub> implies that physical and biological seed priming techniques may work in concert to produce better early-stage ion compartmentalization. Furthermore, greatly affecting NHX1 expression was salinity. At 8 dS/m (S<sub>2</sub>), with 3.11-fold, followed by 12 dS/m (S<sub>6</sub>), then 4 dS/m (S<sub>1</sub>) at 1.87-fold, and lastly 0 dS/m (S<sub>0</sub>) at 1.21-fold. These findings show a dose-dependent transcriptional activation of the gene in response to higher Na<sup>+</sup> concentrations, which fits the function of

NHX1 in vacuolar salt sequestration to minimize harmful effects, as shown in more recent investigations. Under 4.08-fold, analysis of the interaction between seed treatment and salinity level revealed that the T<sub>3</sub>-S<sub>2</sub> combination (plasma + seaweed extract under 8 dS/m) obtained the highest expression level followed closely by T<sub>3</sub>-S<sub>3</sub> at 3.87-fold. On the other hand, the T<sub>0</sub>-S<sub>0</sub> interaction (untreated seeds under non-saline circumstances) revealed the lowest expression at 1.00-fold, therefore underlining the inducible character of this gene under stress conditions and the amplifying effect of seed priming in modifying such responses. Ma *et al.* (2021), who observed elevated NHX1 expression in wheat under mild salt stress when seeds were treated with plasma, complement our findings by connecting with greater salt tolerance and development performance. Al-Solami *et al.* (2022) similarly showed that using seaweed-based biostimulants raised NHX1 and SOS1 genes in tomato and barley, thereby improving ionic balance under salinity. Rahimi *et al.* (2023) verified in another investigation that the synergistic mix of abiotic and biotic elicitors enhanced gene expression patterns associated to ion transport and stress tolerance in maize. These analogues confirm the idea that NHX1 is a molecular marker of salt tolerance and that pre-sowing therapies like as cold plasma and seaweed extracts may efficiently control its expression to facilitate early-stage adaptation. Combining such strategies in saline agriculture might provide workable means to increase crop resilience under soil salinization caused by climate change.

### Gene expression of APX1 (Oxidative stress response gene)

The applied seed treatments and salinity levels clearly affected the expression of APX1 (Ascorbate Peroxidase 1)—a fundamental gene in the antioxidant defense system. Reaching 3.18-times compared to the control ( $T_0$ ), which was normalized at 1.00-fold, the combined plasma and seaweed extract treatment ( $T_3$ ) produced the greatest gene expression. Treatments with seaweed extract alone ( $T_2$ ) and plasma alone ( $T_1$ ) reported correspondingly lower expression levels at 2.56-fold and 2.11-fold respectively (Figure 4). The observed increase of APX1 in plasma-treated seeds is compatible with results showing that cold plasma functions as a priming agent via oxidative stress signalling, hence activating antioxidant gene expression (Zhang et al., 2020). Likewise, by adjusting the antioxidant network and therefore enhancing redox equilibrium, seaweed extract—rich in phenolics, flavonoids, and natural antioxidants—has been shown to increase the transcription of stress-responsive genes (Ashraf & Harris, 2021). This mix of physical elicitation and physiological stimulation most certainly produces the synergistic action in  $T_3$ . APX1 expression raised in a dose-dependent way with increasing salt concentrations, peaked at  $S_3$  (12 dS/m) with a 3.21-fold rise, followed by  $S_2$  (8 dS/m),  $S_1$  (4 dS/m), and the lowest in  $S_0$  (0 dS/m) at 1.86-fold. This expression pattern emphasizes the important function of the gene in reducing oxidative stress under salt by means of hydrogen peroxide enzymatic breakdown. These findings are consistent with previous studies showing the vital role APX1 plays in preserving cellular redox stability under circumstances of salt stress (Hasanuzzaman et al., 2023). Treatment and salinity interacted to show that the  $T_3$ - $S_3$  combination (plasma + seaweed at high salinity) achieved the largest APX1 expression at 3.88-fold, followed by  $T_3$ - $S_2$  with 3.73-fold. By contrast, at 1.00-fold the  $T_0$ - $S_0$  control treatment showed the lowest expression. These findings imply that pre-sowing stimulation efficiently "primes" the plant to respond more robustly to oxidative stresses experienced during early development under salt. Our results align with Li et al. (2021), who reported that enhanced salt stress tolerance was matched with higher transcription of antioxidant genes including APX1 by plasma-treated maize seedlings. Likewise, El-Mahdy et al. (2022) under drought and salt stress, exogenous administration of seaweed extract improved APX and CAT gene expression in wheat seedlings, thereby supporting the function of marine bio stimulants in molecular stress priming. Stronger activation of ROS-scavenging genes in barley under saline irrigation was shown in another investigation by Niazi et al. (2023) combining abiotic (plasma) and biotic (natural extract). These data together highlight the need of combining physical and biological seed treatments to upregulate critical antioxidant defense genes including APX1, thereby improving wheat's potential to reduce oxidative stress during germination and early development in salty conditions.

### Conclusion

Under salty circumstances, the combined use of cold atmospheric

plasma and seaweed extract ( $T_3$ ) greatly enhanced wheat seed germination, early seedling development, enzyme activity, and stress-related gene expression. At 8 dS/m salinity, where  $T_3$  improved germination to 89.42%, lowered mean germination time to 3.56 days, and over 3-fold elevated P5CS, NHX1, and APX1 genes, performance was greatest. This combined therapy turned off most detrimental effects of salt stress and triggered important physiological and molecular defenses. For seed priming in salt-affected soils, the method presents a potential, environmentally friendly solution.

### DECLARATIONS

#### Author contribution statement

Conceptualization: J.J.K. and A.T.Y.; Methodology: H.S.H.; Software and validation: H.S.H. and A.N.F.; Formal analysis and investigation: A.N.F.; Resources: J.J.K. and A.T.Y.; Data curation: H.S.H.; Writing—original draft preparation: A.N.F.; Writing—review and editing: J.J.K. and A.T.Y.; Visualization: A.N.F.; Supervision: J.J.K.; Project administration: J.J.K.; Funding acquisition: Not applicable. All authors have read and agreed to the published version of the manuscript.

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**Ethics approval:** This study did not involve any animal or human participants; thus, ethical approval was not applicable.

**Consent for publication:** All co-authors gave their consent to publish this paper in AAES.

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