



## ORIGINAL: Cold Plasma Effects on Seed Priming of Sage (*Salvia officinalis* L.)

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### ABSTRACT

Cold plasma is an emerging physical technology for seed priming; however, its effects on sage (*Salvia officinalis* L.) remain poorly characterized. This study investigated the effects of dielectric barrier discharge (DBD) argon plasma on germination indices, seedling growth, and biochemical traits of sage seeds. A completely randomized design with four treatments (control, 3, 5, and 10 min plasma exposure) and three replications was employed.

Plasma treatment significantly ( $p < 0.001$ ) enhanced all germination traits. Germination percentage increased from 75.67% (control) to 94.00% (10 min), while germination rate rose from 3.20 to 4.90. Mean germination time decreased from 6.20 to 4.00 days. Radicle and plumule lengths increased by 92% and 64%, respectively, at 10 min. Soluble carbohydrates and total proteins reached their maximum values at 5 min (17.47 and 11.20 mg/g FW), showing 44% and 43% increases over the control. Vegetative growth parameters, including root length, shoot length, and leaf number, were also maximized at 5 min. Seedling vigor index showed a remarkable increase from 16.74 (control) to 45.08 (10 min), representing a 2.7-fold improvement. The 5-min treatment was optimal for vegetative growth and biochemical accumulation, while 10-min was superior for germination traits. These improvements are attributed to enhanced seed coat permeability, increased enzyme activity, and improved reserve mobilization induced by plasma-generated reactive species. Therefore, cold plasma is proposed as a non-chemical, eco-friendly priming method for medicinal plant cultivation.

## Introduction

In recent years, the application of cold plasma (non thermal plasma) as a novel physical method for seed priming and germination enhancement has attracted considerable attention (1,2). Cold plasma, through the generation of reactive oxygen and nitrogen species (RONS) and

physicochemical modifications of the seed surface, increases coat permeability and facilitates water uptake (3,4). Recent studies on various species, including those of the genus *Salvia*, have demonstrated that plasma duration and gas type exert a determining influence on outcomes (2). For sage (*Salvia*

spp.), it has been reported that plasma can improve germination rate and uniformity, and increase germination percentage under both normal and stress conditions (2). Microscopic examinations reveal that plasma induces surface modifications (etching) and pore opening on the seed coat, which are directly associated with enhanced water absorption (2). From a biomolecular perspective, plasma can stimulate the activity of enzymes involved in the mobilization of seed reserves (such as  $\alpha$  amylase) and the expression of germination related genes (5). Beyond germination improvement, reports indicate increased early seedling growth and enhanced tolerance to environmental stresses following plasma priming in experimental samples (6,7).

Research on other species has demonstrated similar results. For instance, in soybean (*Glycine max*), plasma treatment of seeds resulted in increased germination indices and root and shoot growth (4,8). In tomato (*Solanum lycopersicum*), cold plasma stimulated the expression of defense genes and increased antioxidant enzyme activity (6,5). In guar (*Cyamopsis tetragonoloba*), plasma priming enhanced germination rate, root and shoot length, and dry weight under salinity stress conditions (3,7). Additionally, in *Prosopis koelziana*, plasma seed treatment improved germination rate even under high salinity and reduced lipid peroxidation (1). However, seed responses to plasma are highly dependent on species, seed conditions (moisture content, seed age), and plasma parameters (gas type, power, exposure time) (5). Recent studies emphasize the need to integrate microscopic, biochemical, and molecular criteria for interpreting plasma mechanisms of action (2,9).

For sage (*Salvia officinalis* L.), as an economically and medicinally important species, optimizing plasma protocols can play a significant role in enhancing cultivation yield and active ingredient quality (2). Furthermore, the integration of plasma with novel data analysis approaches and machine learning for predicting optimal parameters has garnered attention (5). Future research should

focus on parameter standardization, field trials, and investigation of long term effects on plant chemical composition and economic returns (6,9). Reports on the effects of cold plasma on seed germination across various plant species remain limited; therefore, investigating the effects of cold plasma treatment on seed germination and growth of sage is essential for improving seed germination performance. Despite the growing body of evidence on cold plasma effects in various crops, comprehensive studies simultaneously evaluating germination, vegetative growth, and biochemical responses in *Salvia officinalis* L. are lacking. Moreover, the optimal exposure duration for this medicinally and economically important species has not been established. Therefore, the present study was designed to fill this gap by systematically investigating the time-dependent effects of DBD argon plasma on multiple physiological and biochemical parameters in sage seeds. We hypothesized that DBD argon plasma treatment would enhance germination indices, seedling growth, and biochemical traits of *S. officinalis* seeds in a duration-dependent manner, with intermediate exposure times (5–10 min) eliciting optimal responses through improved seed coat permeability and enhanced metabolic activity.

## Material and Methods

### Seed Material

Sage seeds were obtained from the Agricultural and Natural Resources Research Center of Mazandaran Province, Iran. The seeds were organically produced and collected in 2024.

### Experimental Procedure

Seeds were directly treated using a dielectric barrier discharge (DBD) plasma device at different exposure durations and subsequently irrigated with tap water. For plasma treatment, dry seeds free from any surface contaminants were directly exposed to the treatment. Seeds of approximately uniform size were selected. A specified number of seeds from each sample were placed in the plasma device under predetermined

conditions. Seeds treated under identical plasma conditions were considered as one replicate.

### **Dielectric Barrier Discharge Plasma Treatment**

Seed treatments using DBD plasma were conducted on October 26, 2024. Twenty seeds, considered as one replicate, were placed on the dielectric surface of the plasma device. The gas inflow rate was 15 L/min, and the input voltage was adjusted to 70 V using a variac transformer. The DBD system operated at a frequency of 5 kHz with a 2 mm gap between the dielectric electrodes. Upon voltage application between the two electrodes, uniform plasma was generated between them, and seeds were exposed to plasma treatment. Treatments consisted of exposure to DBD plasma for different durations of 3, 5, and 10 minutes. Each treatment was performed in three replications, with 30 seeds per replicate subjected to identical plasma conditions. The DBD plasma system (Model: DBD-200, NikPazhohan Co., Tehran, Iran) consisted of two parallel circular aluminum electrodes (diameter: 8 cm) covered with a dielectric layer of quartz glass (thickness: 2 mm). The high-voltage electrode was connected to a power supply (0-100 V, 50 Hz) through a variac transformer, while the ground electrode was fixed. The discharge gap between the electrodes was maintained at 2 mm using Teflon spacers.

### **Germination Test**

The germination test aims to determine the maximum germination capacity of seeds, which can be used to compare seed quality among different lots and estimate the amount of seed required for field planting.

### **Seed Germination Assay**

Plastic containers and the germination medium, consisting of two layers of filter paper, were sterilized using a UV tunnel. Three replicates of 20 seeds were considered for each treatment. Treated seeds were placed on the two-layer filter paper moistened with tap water within the plastic containers. Additionally, a control group consisting of untreated seeds was included in the

experiment. The containers were then incubated in a germination chamber at 70% relative humidity under a constant temperature of 25°C, with a photoperiod of 16 h light and 8 h darkness. Germination progress was monitored daily. After the emergence of the first radicle, seeds were removed from the germinator daily, and the number of germinated seeds was counted and recorded. Radicle emergence of 2 mm was considered the criterion for seed germination. At the end of the experiment, traits related to seed germination were measured and calculated.

### **Measurement of Germination Traits**

Following the final germination count, radicle and plumule lengths were measured using a digital caliper (accuracy: 0.01 mm). Subsequently, germination percentage, germination rate, mean germination time, germination index, seedling length, and seedling vigor were calculated using the following formulas (according to ISTA, 2024):

Germination percentage (GP) =  $(n/N) \times 100$

Germination rate (GR) =  $\Sigma(Nt/t)$

Mean germination time (MGT) =  $\Sigma(Ni \times Di) / n$

Germination index (GI) =  $\Sigma(Di \times ni) / N$

Seedling length (SL) = Radicle length + Plumule length

Seedling vigor index (SVI) = Germination rate  $\times$  Seedling length

Where:

- n = total number of germinated seeds at the end of the test
- N = total number of seeds sown per container (20 seeds)
- Nt = number of newly germinated seeds at day t
- Ni = number of germinated seeds on day i
- Di = number of days after the start of the test until the i-th count

### **Measurement of Biochemical and Vegetative Parameters**

This experiment was conducted in a pot culture system. Five germinated seeds with uniform radicle length (approximately 5 mm) were transplanted into each pot at a depth of 1 cm. The pots were maintained in a growth chamber under controlled conditions ( $25 \pm$

2°C, 16 h light/8 h dark photoperiod, 65-70% relative humidity). Pots measuring 8 × 7 cm were prepared, and the soil mixture in each pot consisted of 50% perlite and 50% cocopeat. Germinated seeds were subsequently transferred to the pots. Pots were irrigated every three days and maintained for five weeks. Once per week, pots received B&D nutrient solution. Additionally, 1 mM KNO<sub>3</sub> solution was used as a nutrient supplement. After five weeks, vegetative traits were measured. Samples were then frozen in liquid nitrogen and transferred to the laboratory for biochemical analyses.

#### Measurement of Vegetative Traits

Vegetative traits measured in this experiment included: root length, shoot length, leaf number, and leaflet number. Root and shoot lengths were measured using a ruler.

#### Total Protein Assay

After five weeks of growth, the aerial parts (shoots and leaves) of the seedlings were carefully harvested, separated from the roots, washed thoroughly with distilled water, blotted dry with filter paper, and immediately frozen in liquid nitrogen. The samples were then stored at -80°C until biochemical analyses. For both protein and carbohydrate assays, the frozen aerial tissues were ground to a fine powder and used for extraction.

For protein extraction, 0.02 g of frozen aerial tissue was ground in a pre-chilled porcelain mortar with 0.5 mL of cold extraction buffer (50 mM phosphate buffer, pH 7.2, containing 1 mM EDTA, 1 mM PMSF, and 1% PVP). The homogenate was centrifuged at 4°C for 20 min, and the resulting supernatant was used for protein determination. Total soluble protein content was measured using the Bradford method (1976), with bovine serum albumin as the standard. Absorbance was read at 595 nm, and protein concentration was calculated based on a standard curve and expressed as mg/g fresh weight.

#### Soluble Carbohydrate Assay

Soluble carbohydrates were extracted from 0.05 g of frozen aerial tissue using 2.5 mL of 80% ethanol. The mixture was incubated in a water bath at 95°C for 60 min. After

centrifugation, the supernatant was collected, and soluble carbohydrate content was determined using the anthrone reagent method described by Fales (1951). Absorbance was read at 625 nm, and the concentration of soluble carbohydrates was calculated using a glucose standard curve and expressed as mg/g fresh weight.

#### Statistical Analysis

The experiment was conducted as a completely randomized design (CRD) with four treatments (control, 3, 5, and 10 min plasma exposure) and three replications. All data were expressed as mean ± standard error (SE). Prior to analysis, the normality of data distribution was assessed using the Shapiro-Wilk test, and the homogeneity of variances was verified using Levene's test. When assumptions were met, data were subjected to one-way analysis of variance (ANOVA) using SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA). When a significant F-test was detected ( $p < 0.05$ ), mean comparisons were performed using Fisher's least significant difference (LSD) test at the 5% probability level.

## Results

#### Germination Traits

Analysis of variance revealed that cold plasma treatment had a highly significant effect ( $p < 0.001$ ) on all measured germination traits (Table 1). In general, plasma exposure progressively improved germination parameters with increasing treatment duration. The 10-min treatment consistently produced the highest values for germination percentage, germination rate, germination index, and the shortest mean germination time. Compared to the control, these represented increases of 24.2%, 53.1%, and 59.1% in germination percentage, germination rate, and germination index, respectively, coupled with a 35.5% reduction in mean germination time.

Similarly, radicle and plumule lengths showed marked improvements following plasma exposure. The 10-min treatment produced the longest radicles and plumules, resulting in a total seedling length that was

nearly double the control value. Consequently, the seedling vigor index increased dramatically from 16.74 in the control to 45.08 in the 10-min treatment, representing a 2.7-fold enhancement (Table

1). These findings indicate that plasma treatment not only improved germination kinetics but also promoted early seedling growth, leading to significantly more vigorous seedlings.

**Table 1.** Effect of cold plasma treatment on germination traits of sage seeds

| Treatment | Germination (%) | Germination rate | Mean germination time (days) | Germination index | Radicle length (cm) | Plumule length (cm) | Seedling length (cm) | Vigor index    |
|-----------|-----------------|------------------|------------------------------|-------------------|---------------------|---------------------|----------------------|----------------|
| Control   | 75.67 ± 2.08 d  | 3.20 ± 0.10 d    | 6.20 ± 0.10 a                | 60.33 ± 1.53 d    | 2.13 ± 0.06 d       | 3.10 ± 0.10 d       | 5.23 ± 0.15 d        | 16.74 ± 0.81 d |
| 3 min     | 81.67 ± 1.53 c  | 3.80 ± 0.10 c    | 5.20 ± 0.10 b                | 74.00 ± 1.00 c    | 3.00 ± 0.10 c       | 3.60 ± 0.10 c       | 6.60 ± 0.20 c        | 25.08 ± 1.18 c |
| 5 min     | 90.33 ± 1.53 b  | 4.50 ± 0.10 b    | 4.40 ± 0.10 c                | 88.33 ± 1.53 b    | 3.67 ± 0.06 b       | 4.33 ± 0.06 b       | 8.00 ± 0.10 b        | 36.00 ± 0.81 b |
| 10 min    | 94.00 ± 1.00 a  | 4.90 ± 0.10 a    | 4.00 ± 0.10 d                | 96.00 ± 1.00 a    | 4.10 ± 0.10 a       | 5.10 ± 0.10 a       | 9.20 ± 0.20 a        | 45.08 ± 1.35 a |
| p-value   | < 0.001         | < 0.001          | < 0.001                      | < 0.001           | < 0.001             | < 0.001             | < 0.001              | < 0.001        |
| LSD       | 2.15            | 0.18             | 0.25                         | 2.41              | 0.15                | 0.17                | 0.32                 | 1.85           |

Values are presented as mean ± SE (n = 3). Different letters within each column indicate significant differences according to LSD test (p < 0.05).

### Vegetative Growth Traits

The analysis of variance indicated that plasma treatment significantly affected all vegetative growth parameters, including root length, shoot length, leaf number, and leaflet number (p < 0.01 for leaf number; p < 0.001 for the other traits; Table 2).

The greatest root length (7.20 cm) and shoot length (4.47 cm) were observed in the 5-min plasma treatment, representing increases of 41.2% and 50.5%, respectively, compared to the control (5.10 cm and 2.97 cm). Although

the 10-min treatment also enhanced both parameters (6.23 cm and 3.83 cm, respectively), the 5-min treatment consistently produced the maximum values. Leaf number increased from 3.67 in the control to 6.33 in the 5-min treatment, while leaflet number rose from 7.67 (control) to 13.00 under the same plasma condition. Notably, the 3-min treatment also improved all vegetative traits compared to the control, though to a lesser extent than the 5-min treatment (Table 2).

**Table 2:** Effect of cold plasma treatment on vegetative growth traits of sage seedlings after five weeks

| Treatment  | Root length (cm) | Shoot length (cm) | Leaf number per plant | Leaflet number |
|------------|------------------|-------------------|-----------------------|----------------|
| Control    | 5.10 ± 0.10 d    | 2.97 ± 0.06 d     | 3.67 ± 0.33 c         | 7.67 ± 0.33 c  |
| 3 min      | 5.97 ± 0.06 c    | 3.67 ± 0.06 c     | 5.00 ± 0.58 b         | 10.67 ± 0.88 b |
| 5 min      | 7.20 ± 0.10 a    | 4.47 ± 0.06 a     | 6.33 ± 0.33 a         | 13.00 ± 0.58 a |
| 10 min     | 6.23 ± 0.06 b    | 3.83 ± 0.06 b     | 4.67 ± 0.33 b         | 9.00 ± 0.58 b  |
| p-value    | < 0.001          | < 0.001           | < 0.01                | < 0.001        |
| LSD (0.05) | 0.21             | 0.14              | 0.96                  | 1.52           |

Values are presented as mean ± SE (n = 3). Different letters within each column indicate significant differences according to LSD test (p < 0.05).

### Biochemical Traits

Plasma treatment had a highly significant effect (p < 0.001) on both soluble carbohydrate and total protein contents (Table 3). All plasma treatments increased these biochemical parameters compared to the control, with the highest values consistently observed in the 5-min treatment.

Soluble carbohydrate content increased progressively from 12.13 mg/g FW in the control to 17.47 mg/g FW in the 5-min plasma treatment, representing a 44.0% increase. Similarly, total protein content rose from 7.83 mg/g FW in untreated seeds to 11.20 mg/g FW in the 5-min treatment, corresponding to a 43.0% enhancement. Although the 10-min

treatment also elevated both parameters (15.13 and 9.67 mg/g FW, respectively), the values were slightly lower than those obtained at 5 min, suggesting that intermediate exposure duration may be optimal for stimulating metabolic activity (Table 3).

**Table 3:** Effect of cold plasma treatment on biochemical traits of sage seedlings

| Treatment | Soluble carbohydrate (mg/g FW) | Total protein (mg/g FW) |
|-----------|--------------------------------|-------------------------|
| Control   | 12.13 ± 0.31 d                 | 7.83 ± 0.21 d           |
| 3 min     | 14.30 ± 0.36 c                 | 9.10 ± 0.26 c           |
| 5 min     | 17.47 ± 0.25 a                 | 11.20 ± 0.20 a          |
| 10 min    | 15.13 ± 0.40 b                 | 9.67 ± 0.21 b           |
| p-value   | < 0.001                        | < 0.001                 |
| LSD       | 0.73                           | 0.48                    |

### Overall Trends

In summary, the results demonstrated that cold plasma treatment, particularly at durations of 5 and 10 minutes, significantly improved germination indices, vegetative growth, and biochemical traits of sage seeds compared to the untreated control. For most parameters, a clear dose-dependent response was observed, with longer exposure times (up to 10 min) generally producing the greatest improvements in germination traits, while intermediate exposure (5 min) was optimal for vegetative growth and biochemical accumulation. These findings indicate that plasma priming effectively stimulates physiological and metabolic processes during early growth stages of sage, offering a promising non-chemical approach for enhancing seed performance in medicinal plants.

### Discussion

The present study demonstrated that DBD argon plasma treatment significantly improved germination indices, vegetative growth, and biochemical traits of sage (*Salvia officinalis* L.) seeds. Our results revealed that plasma exposure at 5 and 10 min effectively enhanced germination percentage, rate, and seedling vigor while reducing mean germination time. These findings align with previous reports indicating that cold plasma serves as an effective physical priming

technique for stimulating seed germination and early seedling development in various plant species (4,10,2).

The observed improvements in germination traits can be attributed to multiple physicochemical mechanisms induced by cold plasma treatment. First, plasma-generated reactive oxygen and nitrogen species (RONS), along with charged particles and UV photons, interact with the seed coat surface, leading to physical modifications such as etching, micro-cracking, and increased surface roughness (11,12). These structural changes enhance seed coat permeability, facilitating water uptake and gas exchange, which are critical for initiating germination-related metabolic processes. Second, plasma treatment stimulates hydrolytic enzymes ( $\alpha$ -amylase, protease, dehydrogenase) responsible for mobilizing stored reserves (4,5). The increased soluble carbohydrate and total protein contents observed in our study (particularly at 5 min) support this mechanism, indicating enhanced reserve mobilization and metabolic activation.

The significant reduction in mean germination time from 6.20 days (control) to 4.00 days (10-min plasma) suggests that plasma treatment not only increases the final germination percentage but also accelerates germination kinetics, leading to more uniform seedling emergence. This is of practical importance for medicinal plant cultivation, where uniform establishment can reduce production costs and improve crop management. Similar accelerations in germination rate have been reported in soybean (8), sweet basil (10), and guar (3) following cold plasma priming.

Our results also demonstrated that plasma treatment significantly enhanced vegetative growth parameters, including root length, shoot length, leaf number, and leaflet number, with the 5-min treatment producing the maximum values. The increase in root and shoot lengths indicates stimulation of cell division and elongation in meristematic tissues, likely mediated by enhanced energy availability (via increased soluble

carbohydrates) and protein synthesis (via increased total protein content). Furthermore, the increased leaf and leaflet numbers suggest that plasma treatment positively affects apical meristem activity and promotes early vegetative development. These findings are in agreement with studies on tomato (6), pea (13), and green bell pepper (14), where cold plasma treatment resulted in improved shoot and root growth.

From a biochemical perspective, the significant increases in soluble carbohydrates and total proteins in plasma-treated seeds indicate enhanced metabolic activity and reserve mobilization. Soluble carbohydrates serve as immediate energy sources for germination and early seedling growth, while proteins are essential for structural components and enzymatic functions. The highest values for both parameters were observed in the 5-min treatment, suggesting that intermediate exposure duration is optimal for stimulating biosynthetic pathways without causing oxidative damage. The increase in soluble carbohydrates is likely due to enhanced  $\alpha$ -amylase activity, which degrades stored starch into readily metabolizable glucose and maltose (4). Similarly, the increase in total protein content may result from stimulated protein synthesis or enhanced protease activity, which mobilizes storage proteins into free amino acids and peptides (10). This dose-dependent response has been previously documented in pea and zucchini seeds, where longer exposure times had inhibitory effects due to accumulation of reactive species and excessive oxidation (15). Comparisons with previous studies on other *Salvia* species reveal both similarities and differences. Ghodsimaab et al. (2) reported that cold plasma improved germination in *Salvia leriifolia*, but optimal durations varied between species. While they found 5 min effective for *S. leriifolia*, our results showed that 10 min was superior for germination traits in *S. officinalis*. This discrepancy may be attributed to differences in seed coat thickness, dormancy levels, or genetic background between the two species. Additionally, Yadegari (16) demonstrated

hormonal responses in three *Salvia* species, suggesting that plasma-induced changes in phytohormone balance may be species-specific. Our biochemical data (increased soluble carbohydrates and proteins) support this notion, as the magnitude of metabolic stimulation differed between exposure durations, indicating that *S. officinalis* may require longer plasma exposure to achieve maximum germination potential.

The differential responses observed across plasma exposure durations highlight the importance of optimizing treatment parameters for each plant species. In our study, 5-min treatment was optimal for vegetative growth and biochemical accumulation, while 10-min treatment was superior for germination traits. This suggests that germination and early growth may have different sensitivity thresholds to plasma-induced stimuli, possibly due to differential activation of signaling pathways. Similar species-specific and duration-dependent responses have been reported in cumin (17), Camelina (18), and soybean (19), emphasizing the need for empirical optimization of plasma parameters for each crop.

From a mechanistic perspective, the positive effects of cold plasma can be attributed to several interconnected pathways: (1) physical modification of the seed coat, increasing permeability to water and oxygen; (2) generation of reactive species (ROS and RNS) that act as signaling molecules, triggering downstream physiological responses; (3) activation of antioxidant defense systems, reducing lipid peroxidation and maintaining membrane integrity; (4) stimulation of phytohormone biosynthesis (e.g., gibberellins and cytokinins), promoting cell division and elongation; and (5) upregulation of germination-related genes, including those encoding hydrolytic enzymes and stress-responsive proteins (5,4,2). While our study did not directly measure these molecular parameters, the observed biochemical and physiological changes are consistent with these proposed mechanisms.

The results of this study are largely consistent with previous investigations on cold plasma effects on seed germination and seedling growth. For instance, Ling et al. (4) reported that cold plasma treatment increased germination percentage by 25-44% and enhanced antioxidant enzyme activity in rapeseed, which improved seedling drought tolerance. Similarly, Singh et al. (10) demonstrated that cold plasma at optimal power (150 W) increased germination percentage and seedling growth in sweet basil, accompanied by increased water uptake and soluble carbohydrate and protein content. Our findings align with these reports, confirming that cold plasma can effectively stimulate early growth processes by establishing balance in metabolic activities. However, the effectiveness of plasma treatment is highly dependent on multiple factors, including plasma type (DBD, plasma jet, corona discharge), gas composition (argon, helium, nitrogen, air, or mixtures), power density, exposure duration, and seed characteristics (species, moisture content, age, and dormancy status) (5,2). In our study, DBD argon plasma at 70 V with exposure durations of 5-10 minutes proved effective for sage seeds. Future research should investigate the effects of different gases (e.g., nitrogen, oxygen, and helium) and voltage intensities on physiological responses of sage seeds, as well as the long-term effects on plant growth, yield, and secondary metabolite production. The present study has several limitations that should be acknowledged. First, we did not perform scanning electron microscopy (SEM) analysis to directly visualize surface modifications on plasma-treated seeds, which would have provided direct evidence for enhanced permeability. Second, we did not measure enzymatic activities (e.g.,  $\alpha$ -amylase, protease, or antioxidant enzymes) or gene expression levels, which would have elucidated the molecular mechanisms underlying the observed physiological responses. Third, the experiment was conducted under controlled laboratory conditions, and the results may not fully translate to field conditions, where

environmental stressors (drought, salinity, temperature fluctuations) could modulate the effectiveness of plasma priming. Future studies should address these limitations by incorporating SEM imaging, enzymatic assays, molecular analyses, and field trials to validate the practical applicability of plasma technology for sage cultivation.

## Conclusion

Cold plasma treatment (5-10 min) using DBD argon significantly improved germination, early growth, and biochemical traits of *Salvia officinalis* seeds. The 10-min treatment was superior for germination indices, while the 5-min treatment optimized vegetative growth and reserve mobilization. Our findings demonstrate that DBD argon plasma priming is a safe, cost-effective, and environmentally friendly alternative to chemical treatments for enhancing sage seed performance. The 5-min treatment optimally balances germination enhancement and vegetative growth, making it suitable for commercial nursery production. Future research should focus on: (i) optimizing plasma parameters (gas composition, power) for maximum efficacy, (ii) evaluating long-term effects on secondary metabolite production (essential oils, phenolics) under field conditions, (iii) conducting transcriptomic and proteomic analyses to elucidate molecular mechanisms, and (iv) assessing the economic feasibility of plasma priming for large-scale medicinal plant cultivation.

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## Authorship

SR conceived and designed the experiments; FHJ and MSV performed the experiments; SR analyzed the data; FHJ and MSV wrote the original draft; SR reviewed and edited the manuscript. All authors read and approved the final manuscript.

## Conflicts of interest

The authors declare that they have no competing interests.

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