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# VLIV ABIOTICKÝCH A BIOTICKÝCH STRESORŮ NA VLASTNOSTI ROSTLIN 2025

(Sborník recenzovaných vědeckých prací)

## INFLUENCE OF ABIOTIC AND BIOTIC STRESSES ON PROPERTIES OF PLANTS 2025

*(Proceedings of scientific articles)*

**Czech University of Life Science Prague**

**Institute of Forest Ecology SAS**

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# SEED PRIMING WITH PLASMA-ACTIVATED WATER AFFECTS SEED GERMINATION AND SEEDLING GROWTH OF *SALVIA OFFICINALIS*

## *PRIMING SEMEN POMOCÍ PLAZMOU AKTIVOVANÉ VODY OVLIVŇUJE KLÍČENÍ SEMEN A RŮST SEMANÁČKŮ ŠALVĚJE LÉKAŘSKÉ (SALVIA OFFICINALIS)*

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***Summary***

*Salvia officinalis* (sage) is an evergreen perennial herb of the Lamiaceae family. Its aromatic leaves and woody stems have long been used for medicinal and culinary purposes. To promote germination and growth of Salvia officinalis seeds, a seed priming technique using plasma activated water (PAW) was used. This combined treatment improved germination and seedling growth compared to controls. This suggests that PAW priming is an effective method for improving early growth of S. officinalis, potentially offering a sustainable strategy for medicinal plant cultivation. Further experiments will be directed to control the quantity and quality of secondary metabolites.

*Key words: medicinal plants, Salvia officinalis, PAW, seed priming, seed germination, seedling growth*

***Souhrn***

Šalvěj lékařská (*Salvia officinalis*) je stálezelená vytrvalá bylina z čeledi Lamiaceae. Její aromatické listy a dřevnaté stonky se již dlouho používají k léčebným a kulinářským účelům. Pro podporu klíčivosti a růstu semen S. officinalis byla použita technika priming semen za použití plazmou aktivované vody (PAW). Toto kombinované ošetření zlepšilo klíčivost a růst sazenic ve srovnání s kontrolami. To naznačuje, že PAW priming je účinná metoda pro zlepšení raného růstu S. officinalis a potenciálně nabízí udržitelnou strategii pro pěstování léčivých rostlin. Další pokusy budou směřovány na kontrolu množství a kvality sekundárních metabolitů.

*Klíčová slova: léčivé rostliny, Salvia officinalis, PAW, priming semen, klíčení semen, růst semenáčků*

## INTRODUCTION

*Salvia officinalis* is a valuable medicinal and aromatic plant, but its seed germination can face challenges due to the prolonged dormancy and low sensitivity to environmental conditions

/1-3/. Various seed priming techniques, have so far been tested to improve seed germination and seedling growth, including hydropriming and biopriming /1, 4-7/. Hydropriming has been shown to increase the final germination percentage by 25.5% for 12 hours cultivation at temperature of 30 0C, compared to unprimed seeds /1/. Biopriming treatments of seeds with plant hormones, such as gibberellic acid (GA), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and naphthylacetate, could also effectively affect germination rate and seedling vigorous characteristics /2, 6/. However, hydropriming could take longer to treat seeds and higher cost of hormonal/hormone-like chemicals would be used in biopriming. Unfortunately, neither can be used in cost-efficiency enhancements of seed germination and seedling vigor.

Over recent years, PAW was developed into an innovative seed priming technology, showing its synergistic effects of seed priming on enhancing seed germination and vegetative growth in various plants, comprising medicinal herbs /6, 8, 9/. Exposing water to cold plasma can lead to the formation of RONS which contributes to its unique effects on seed priming and nutrition for effective germination and growth tolerance to biotic and abiotic stress /9, 10/. On the other hand, PAW is considered as an eco-friendly alternative to traditional priming methods due to its minimal environmental impact and green chemical nature /9-11/.

Apart from a wide range of applications in agriculture, PAW ability to enhance herbal seed germination and medicinal plant growth still needs further study. Therefore, the present study investigated PAW effects on seed priming for S. officinalis germination and seedling vigor. Future research could optimize PAW treatments for S. officinalis seeds to improve its cultivation and yield.

## MATERIALS AND METHODS

The *Salvia officinalis* L. seeds were purchased from a seed company named Osiva Moravia from the Czech Republic. The seeds were stored at room temperature before being treated with various PAWs that had been exposed to cold plasma for different lengths of reaction time (5, 10, 15, and 20 min, hereafter referred to PAW-5, -10, -15, and -20). The PAWs were prepared using a pin-to-plate plasma setup and offered by our collaborative laboratory.

The method of seed treatment used in the present study for testing the effects of PAW on seed germination and seedling initial growth was formulated based on methodological contribution /12/. Five replicates of 150 seeds were conducted in each treatment (PAW-5, PAW-10, PAW-15 and PAW-20), as well as tap water priming (TW) and untreated (UNT) controls. 30 seeds per replicate were placed on the moist filter papers which were rolled like paper towels for cultivating seeds inside /13/. All replicates were packed in the same plastic bag and transferred to a growth chamber set at 25 0C in the dark. Seed germination and seedling length were measured per day, while the dry/fresh biomass were scaled in 14 days.

The collected data were recorded and calculated by Excel spreadsheet. The statistical analysis was carried out using Python programming (Python 3.13.5), in which the visual (Boxplot, Q-Q plot and Histogram) and mathematic (Shapiro-Wilk) methods were used to test data normality, and data homoscedasticity was checked by Bartlett’s test. If the original sampling distribution appeared non-normal, the bootstrap method was conducted to normalize the data distribution for statistical estimation.

## RESULTS AND DISCUSSION

An experiment was conducted to compare the priming of *S. officinalis* seeds with PAW-5, PAW-10, PAW-15, and PAW-20 to TW hydropriming and UNT controls. The seed germination was observed and recorded over a period of 14 days in cultivation. The change in germination percentage was calculated and summarized in Table 1, to qualify and quantify the effects of PAW on seed priming for the enhanced tolerance to abiotic stress. The results showed the seed germination in all treatments increased notably by 39% in PAW-5, 17% in PAW-10, 29% in PAW-15, and 35% in PAW-20, compared to UNT control (Table 1). By comparison to hydropriming control, the germination percentages were higher in PAW-5 (61%), PAW-15 (51%), and PAW-20 (57%) than in TW (49%), only in addition to PAW-10 (39%). In summary, PAW-5 enhanced the germination performance due to the rich reactive oxygen species (ROS) produced when water was exposed to plasma within 5 min /14/. The ROS have been reported to stimulate seed metabolism, break dormancy, and improve stress tolerance /15/. Furthermore, the prolonged time of plasma exposure to water, like PAW-10, PAW-15 and PAW-20, can generate nitrites, nitrates, and ammonia etc. reactive nitrogen species (RNS), which provide nitrogen nutrients for seed germination /16/. However, more nitrites than nitrates could be detected in PAW-10, resulting in inhibition of seed initial metabolism during germination /17/.

In the early seedling growth, both root and shoot lengths were measured separately using the ImageJ software scale. The total seedling length was obtained by summing the length values of root and shoot. The application of PAW treatment to seed priming enhanced seedling and shoot lengths, but just a slight decrease in root length was observed as compared to UNT (Table 1). The enhancement of seedling and shoot lengths could be attributed to RONS present in PAW, which played a crucial role in facilitating cell division, improving water and nutrient uptake, and enhancing enzymatic activity /18/. The best growth of seedling and shoot in length was obtained in PAW-5, while the poorest one was found in PAW-10, which was in line with the germination tests.

*Table 1: Root length (RL), shoot lengths (SL) and total seedling length (TSL) after the treatments of PAW-5, PAW-10, PAW-15, PAW-20 and both controls – TW, UNT. Means and SE are given. According to Tukey's test, different letters indicate a significant difference at 95% confidence.*

| Treatments | Parameters | | | | | |
|---|---|---|---|---|---|---|
| | RL mean±SE (mm) | Tukey test | SL mean±SE (mm) | Tukey test | TSL mean±SE (mm) | Tukey test |
| TW | 52.54±3.61 | a | 27.38±3.80 | a | 78.34±7.02 | a |
| UNT | 52.97±3.90 | a | 26.44±3.49 | a | 78.06±6.14 | a |
| PAW-5 | 51.85±3.47 | a | 32.06±2.72 | a | 82.79±5.77 | a |
| PAW-10 | 45.02±3.64 | a | 31.16±3.18 | a | 72.77±6.36 | a |
| PAW-15 | 49.23±2.81 | a | 31.24±2.18 | a | 78.41±4.85 | a |
| PAW-20 | 52.00±3.17 | a | 28.21±2.50 | a | 78.89±5.34 | a |

PAW has been constantly reported to show significant potential in enhancing both the fresh and dry biomass of various seedlings across different plant species /19, 20/, which was also proved by the herbal plant, *S. officinalis*. As shown in Fig 1, both fresh weight (FW) and dry weight (DW) of *S. officinalis* biomass increased consistently in treatments of PAW-5 (FW:

860 mg; DW: 120 mg), PAW-10 (FW: 490 mg; DW: 75 mg), PAW-15 (FW: 630 mg; DW: 85 mg), PAW-20 (FW: 715 mg; DW: 90 mg) and TW (FW: 550 mg; DW: 80 mg) control, relative to UNT (FW: 260 mg; DW: 40 mg) control. Based on these findings, PAW-5 was the most effective enhancer of early seedling growth in *S. officinalis*, while the biomass in PAW-10 still grew less than that in TW control.

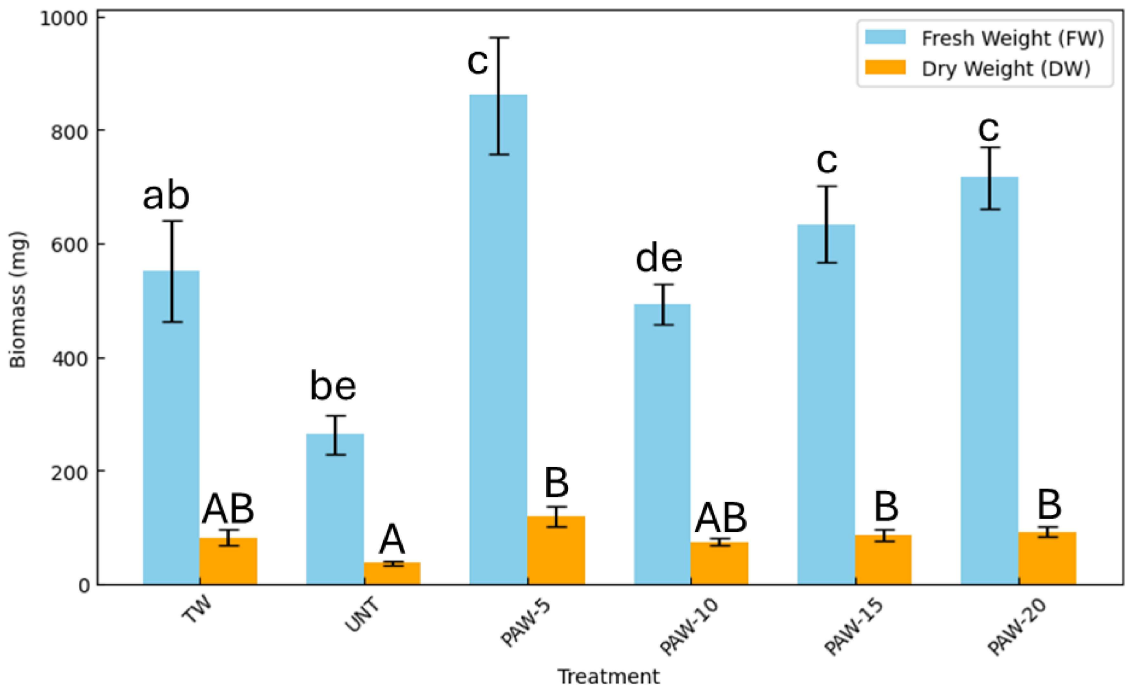


*Figure 1: Fresch weight (FW) and dry weight (DW) of Salvia officinalis biomass after treatment (TW, UNT, PAW-5, PAW-10, PAW-15, PAW-20); mean and SE are given. According to Tukey's test, different letters indicate a significant difference at 95% confidence.*

## CONCLUSION

The present study showed that some seed growth parameters (seed germination, seedling elongation and fresh/dry biomass) of *Salvia officinalis* were enhanced by PAW treatments. The PAWs with different exposure time were used to test equal quantity of seeds and compared to observe germination percentage, seedling (root and shoot) length, and fresh and dry biomass weight. Compared to UNT control, all PAW treatments improved the initial growth quality of *S. officinalis*. In contrast to TW control, however, PAW-10 proved less effective than the control and the other treatments still showed higher potency. The experimental results revealed that PAW priming could also stimulate the growth potential of medicinal plants in addition to only enhancement towards agricultural crops, potentially offering a sustainable insight into an effective and ecofriendly cultivation of medicinal plants.

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