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Edited by G. D. Megersa, E. Maťaš, J. Országh, P. Papp, Š. Matejčík

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POSTER PRESENTATIONS

EFFECT OF PLASMA-ACTIVATED WATER ON PHYSIOLOGICAL PARAMETERS IN BEAN PLANTS (*PHASEOLUS VULGARIS*)

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Our study explores the effects of plasma-activated water (PAW) used for seed priming, foliar application, and their combination on key physiological parameters of bean plants *Phaseolus vulgaris* (hybrid Petronilla) cultivated over three months under field conditions. The findings reveal that PAW treatments significantly enhanced the leaf area, the chlorophyll concentration, and the activity of selected antioxidant enzymes, demonstrating its potential to improve plant growth and stress resilience. These results highlight the effectiveness of PAW as a sustainable agricultural intervention to optimize crop performance in field conditions.

1. Introduction

The growing global population highlights the urgent need for sustainable food production. Modern agriculture heavily relies on commercial fertilizers, with nitrogen being a critical nutrient for plant growth [1, 2]. However, emerging physical methods like static magnetic fields, pulsed electric fields, and cold atmospheric gas plasmas offer innovative and potentially sustainable solutions, though their effects on plants require further investigation [3, 4]. One promising development a source of nitrogen is plasma-activated water (PAW), generated by electrical discharges in contact with water. Its chemical composition, including reactive oxygen and nitrogen species (RONS) like hydrogen peroxide (H_2O_2), nitrites (NO_2^-), and nitrates (NO_3^-), varies based on the plasma discharge type, interaction method, and water buffering capacity [5]. Hydrogen peroxide enters plant cells via facilitated diffusion through aquaporins, playing a crucial role in plant cells and tissues signalling, while NO_2^- and NO_3^- act as essential nitrogen sources, transported through specific transporters [6–9]. These properties position PAW as a sustainable, eco-friendly alternative to traditional fertilizers. This study explores the effects of different PAW applications, such as seed priming and foliar treatments, on bean plants. We examined its impact on key morphological and physiological parameters, including leaf area, concentration of chlorophylls, soluble phenolics, and selected antioxidant enzymes activity in young leaves of adult plants.

2. Methodology

Bean plants were cultivated over a three-month period in 90-liter pots located in the open-air atrium of the Faculty of Natural Sciences at Comenius University Bratislava. Each pot contained nine plants, and the study included the following experimental treatments: the control, seeds primed with PAW, the foliar application of PAW, and the combination of seed priming and foliar application. For priming, seeds were immersed in PAW for 8 hours. Bean seeds were soaked in plasma-activated water under controlled conditions, then air-dried before sowing. After drying seeds were sown directly into the pots. This treatment aimed to enhance germination and early plant growth. Foliar treatments were applied weekly, with each seedling receiving 3 mL of PAW per application. PAW was sprayed on Bean plants, starting when plants had three true leaves and continuing until flowering. Applications were done in early morning using a hand pump pressurized bottle to ensure even coverage.
PAW was prepared by batch treatment of regular tap water (pH=7.9) with multiple parallel transient spark (TS) discharges [10], typically 170 ml of tap water was treated by 17 discharges during 10 min.

Schematic diagram of TS plasma is shown in Fig 1. Each TS was set at approximately 1 kHz repetition frequency of the pulses and 1 cm distance of the power high voltage electrodes from the water surface. The PAW was applied to the plants fresh, within 10 min from the treatment. In such conditions its chemical characteristics were typically as follows: pH=7.5, concentrations of H_2O_2 = 452 µM , O_3=10 µM, NO_2^- = 980 µM, NO_3^- = 1.7 mM.
After three months, young leaves were sampled to evaluate the effects of plasma-activated water (PAW) on various physiological parameters. Leaf area was measured using the Image J software. Chlorophyll concentration was determined following the protocol described by Lichtenthaler [11]. Soluble phenolic content was quantified using the method outlined by Ainsworth and Gillespie [12]. In addition, the activity of the key antioxidant enzymes, including superoxide dismutase (SOD) [13], ascorbate peroxidase (APX) [14], guaiacol peroxidase (G-POX) [15] and catalase (CAT) [16] was monitored. Statistical analysis was conducted using Statgraphics Centurion XV version 15.2.05 (StatPoint, Inc., Warrenton, VA, USA) and Microsoft Excel (Microsoft Office). Treatment effects were assessed via analysis of variance (ANOVA), with single-step multiple comparisons performed, and the least significant difference (LSD) test applied at a significance level of $P < 0.05$.

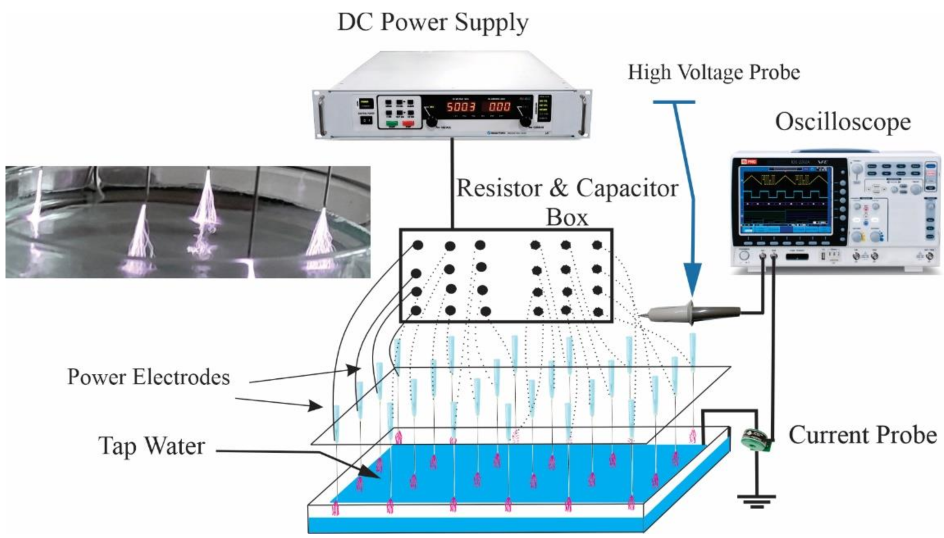

Fig. 1. Schematic diagram of transient spark discharge.

3. Results and Discussion

The foliar application of PAW significantly enhanced the leaf area of young bean plants, with a notable increase compared to the control. The combination of seed priming and foliar application resulted in the most pronounced differences, producing the largest leaves. A positive effect of PAW was also observed in chlorophyll concentration, a key indicator of photosynthetic activity, with a significant increase observed across all treatments compared to the control (Fig. 2). However, in the priming & spraying treatment, chlorophyll *a* concentration was significantly lowered compared with other PAW treatments. PAW acts as a nitrogen source for plants. Nitrate, essential for photosynthesis, supports chlorophyll synthesis in leaves contributing to their growth and development. This highlights the potential of PAW to enhance both physiological and biochemical parameters critical for plant productivity.

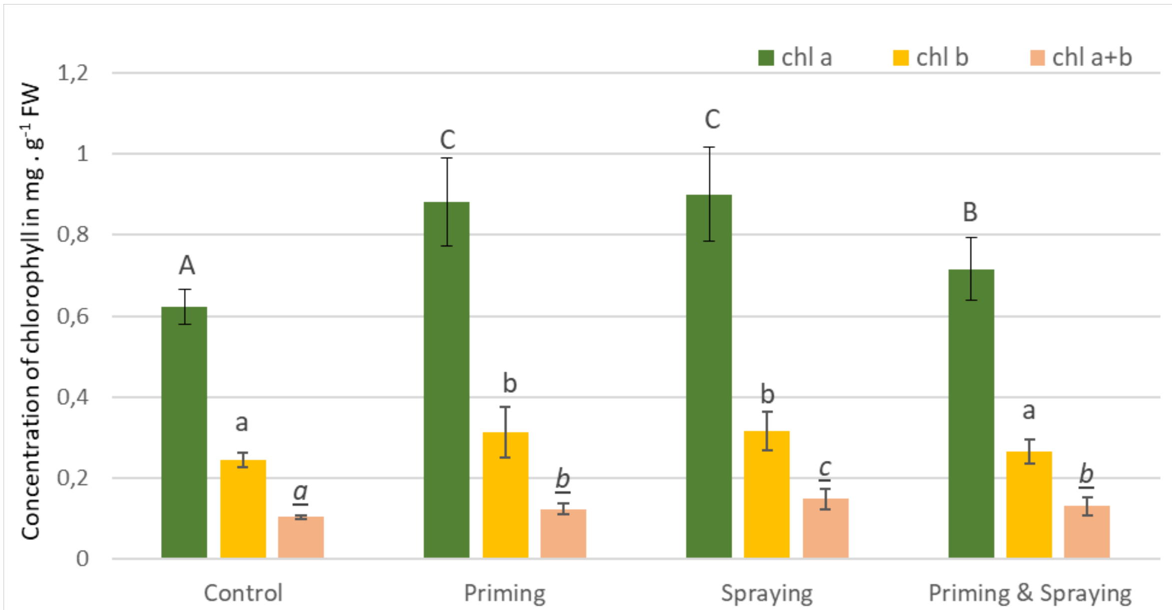

Fig. 2. Chlorophyll concentration in mg . g^{-1} fresh weight (FW) in young bean leaves. Different letters represent statistically significant differences among the treatments at $P \leq 0.05$.

Antioxidant enzymes are essential components of a plant's defence mechanism against environmental stresses. Superoxide dismutase is an essential enzyme in plant defence mechanisms against oxidative stress. Superoxide dismutase catalyses the dismutation of superoxide radicals into H_2O_2, which must subsequently be reduced to water to prevent oxidative damage. This critical step is facilitated by other enzymes in the antioxidant system, such as APX, G-POX and CAT, which exhibit high substrate affinity for H_2O_2. All three applications of PAW resulted in increased SOD activity compared to the control (Fig. 3A). While H_2O_2 plays a signalling role in stress responses, its excessive concentrations result in oxidative damage, compromising cellular integrity. Peroxidases are critical enzymes in detoxifying ROS, catalysing the conversion of H_2O_2 into water through electron donation, thereby protecting cells from oxidative stress [17]. A notable increase in APX activity was observed in seeds primed with PAW, and in the treatments combining seed priming with foliar application (Fig. 3B). Conversely, the foliar application alone did not significantly affect APX activity. In this case, the G-POX enzyme demonstrated the highest activity, suggesting a compensatory mechanism to manage ROS levels under this treatment condition (Fig. 3C). The differing reactions of these two peroxidases can be attributed to their distinct roles and locations: G-POX is a secretory extracellular enzyme, whereas APX is intracellular. Consequently, they operate in different environments and participate in distinct pathophysiological processes. Similarly, CAT activity was elevated in every PAW treatment relative to the control (Fig. 3D), likely due to its role in scavenging the oxidative effects of H_2O_2 present in PAW. Chen et al. [18] previously demonstrated that appropriate nitrogen application enhances the concentrations of SOD and CAT in sweet potato, findings consistent with the results of this study. The elevated levels of these antioxidant enzymes in this experiment suggest an improved tolerance to oxidative stress induced by ROS. During the vegetative stage, plants experience an increased demand for nitrogen to support their rapid growth. The application of PAW during this critical phase may effectively meet this nitrogen requirement, as PAW contains high concentrations of nitrite (NO_2^-) and nitrate (NO_3^-). This nitrogen supply likely contributes to the observed increase in chlorophyll content (Fig. 2) and the enhanced activity of antioxidant enzymes (Fig. 3), which can be attributed to the reactive nitrogen species (RNS) delivered through PAW treatments.

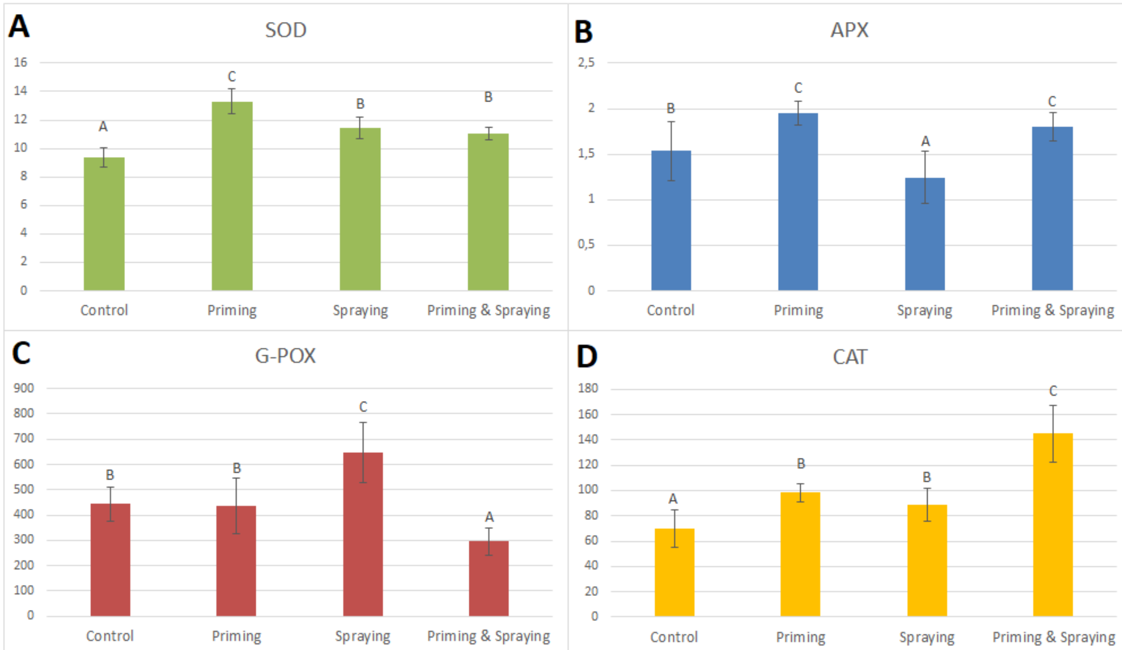

Fig. 3. Activity of SOD (A), APX (B), G-POX (C) and CAT (D) antioxidant enzymes in young leaves of beans plants. Different letters represent statistically significant differences among the treatments at $P \leq 0.05$.

4. Conclusion

Our study demonstrates that PAW significantly enhances adult bean plant growth and oxidative stress tolerance. Plasma activated-water treatments increased the leaf area and chlorophyll content in young bean plants, with the combination of seed priming and foliar application yielding the most significant effects. Furthermore, different application of PAW elevated the activity of almost all monitored key antioxidant enzymes, enhancing the plants' ability to mitigate oxidative damage. By providing reactive nitrogen species (RNS) and nitrates, PAW effectively meets nitrogen demands during critical vegetative growth stages, thereby improving physiological performance and stress resilience.

5. Acknowledgements

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