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Influence of Seed Treated by Plasma Activated Water on the Growth of *Lactuca sativa* L.

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Abstract: The aim of this work was to determine if PAW (Plasma Activated Water) seed treatment and growing conditions could have positive effects on lettuce seedlings and growth. The paper presents the results of a pot experiment on lettuce (*Lactuca sativa* L.) cultivation in greenhouse and field conditions after seed treatment with PAW. The experiment was conducted in two consecutive seasons in 2021 and 2022 and the following growth parameters were measured: head mass, rosette height, rosette width, number of leaves, root mass and root length. As a result of the study, it was found that lettuces grown in the greenhouse from PAW treated seeds had higher results in the first measurement for both cultivars (mass 32.26%, diameter 19.01%, number of leaves 13.49% and height 24.01%), while there were no statistically significant effects on the root system. The lowest results were obtained in untreated and field-grown plants. In addition, plant dry matter was measured and it was found that plants grown from PAW treated seeds had a higher percentage of dry matter (11.51% in 2021, and 11.58% in 2022). It was also found that cultivation in greenhouse resulted in a better quality of plants than the cultivation in the open field.

Keywords: growing conditions; lettuce; PAW; seed treatment

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1. Introduction

Lettuce (*Lactuca sativa* L.) is widely grown vegetable and one of the most important vegetable crops [1]. It is one of the most demanded lettuce crops in both fresh and ready-to-eat markets around the world [2]. The goal of every grower is to achieve higher and better quality yields. At the same time, the elimination of artificial fertilizers, chemical growth promotors and chemical protectants is highly desirable.

A Highly reactive environment with high-energy electrons, radicals, and various gaseous reactive species, including OH, H_2O_2, NO, HNO is generated by various types of atmospheric pressure air discharges generated by cold plasma. PAW (Plasma Activated Water) occurs when plasma comes into contact with water and when these reactive species dissolve in water, creating reactive oxygen and nitrogen species (RONS) [3]. There are many ways in which PAW affects plants: protein synthesis, gene expression, enzyme activity, DNA methylation, DNA demethylation, DNA damage, seed coat morphological and chemical changes, plant hormone balance, germination and seedling growth [4].

The experiments using PAW began in early 21st century. The use of PAW during vegetation also improves resistance to pathogens as well. Plasma treatment of water creates an acidic environment that leads to conductivity and the formation of nitrogen species and reactive oxygen, which change the redox potential. PAW contains nitrites, nitrates, and ammonia which could be nutrients as well as signaling molecules that can promote seed germination and seedling growth [5,6]. These molecules may also help to break dormancy and promote germination. Nitric oxide (NO), one of the reactive nitrogen species produced by plasma, is known to regulate resistance to abiotic and biotic stresses, and plant development [7,8]. According to Thirdumas et al., soaking seeds with PAW not only has an antibacterial effect but also promotes seed germination and plant growth [9]. Nowadays, there are more and more problems in breeding related to climate changes. As a result of global warming there has been a worldwide increase in temperature since 1970 as well as changes in the precipitation regime, leading to several serious consequences for agriculture [10]. Crops are more frequently exposed to stresses of both abiotic and biotic origin, including exposure to unpredictable and extreme climatic events, changes in plant physiology, growing season and phytosanitary hazard, and increased losses of up to 30% and 50% in global agricultural production in the current scenario of rapidly evolving climate change [11]. Predicting the impact of climate change on crop yields is difficult and depends on the crop type and geographic region [12,13] and the type of abiotic stress [14]. Improving crop resilience to stress is critical to maintaining agricultural productivity [15]. Heat waves will occur more frequently, last longer, have higher temperature peaks and longer duration, and begin earlier in the summer than historically [16,17]. Multiple abiotic stress may occur sequentially or simultaneously, generating an important challenge to crop production that requires the analysis of stress combinations in the field [18].

High climate variability and extreme temperature events were experienced in the twentieth century, since the frequency of summer heat waves and spring frost has increased significantly. For plant seedlings, transplanting is an environmentally stressful period as they must adapt to outdoor conditions and quickly establish themselves in the field [14]. Stress to plants caused by adverse environmental conditions can result in lower yields. To meet the requirements of plants, the environment can be artificially modified in such cases, for example in greenhouses [19]. One of the most important components for the development of agriculture has become the greenhouse industry [20]. In a controlled environment in agriculture, the use of greenhouse can help maintain temperature, humidity, and photosynthetically active radiation [21]. To reduce the influence of climate fluctuation, cultivation in protected areas such as greenhouses is often used [22,23]. In addition to climate conditions, seed quality has a significant influence on a good harvest. To achieve high production and productivity rates, the use of high quality seeds is essential [24].

The hypothesis was that seed treatment with PAW and growing conditions (greenhouse and field) will have an effect on the growth and development of two lettuce cultivars and an effect on dry matter. The experiment was conducted with lettuce plants and the parameters evaluated were the visual appearance and growth parameters of the plants (head mass, head height, root mass, root length, number of leaves and rosette diameter) and dry matter. The aim of this work was to study influence of treatment of two lettuce cultivars with PAW on the morphological characteristics of seedlings and lettuce in greenhouse and field conditions.

2. Materials and Methods

The study was conducted with a three-factorial experiment; two varieties of lettuce seeds, PAW treatment for seeds and cultivation under greenhouse and field conditions in four replicates.

2.1. Preparing of Plasma Activated Water

Active species in plasma activated water (PAW) are generated in a plasma reactor based on a single electrode atmospheric pressure plasma jet. The atmospheric pressure plasma jet consists of a quartz tube with outer and inner diameters of 1.5 and 1 mm, respectively, and a copper wire with a diameter of 100 µm that is inserted into the capillary and serves as an electrode. The electrode is powered with a sinusoidal voltage waveform of 28 kHz with a maximum voltage of 12 kV (PVM500-2500 Plasma Power Generator, Information Unlimited), which was selected based on our previous optimization work [25], and the delivered power is typically about 15 W. The discharge is generated in nitrogen gas (99.996% purity), supplied to the capillary at a 500 sccm flow rate. The 215 mL sample in the Berzelius beaker is brought into contact with the plasma jet by placing the liquid surface at a distance of 5 mm from the capillary opening, which insures the comparable production of H_2O_2 and NO_2^- in the PAW. We use a commercial purified pharmaceutical grade water of (Pharmacopoeia Europaea, Ph. Eur. 9) with a pH of 6.5 and a conductivity of 0.98 µS/cm. The treatment time with the nitrogen plasma jet was 40 min. The plasma-activated samples are analyzed immediately after treatment, and several times during their storage. The concentration of NO_2^-, NO_3^- and H_2O_2, as well as the pH of the samples are measured with QUANTOFIX test strips (nitrate/nitrite 500, 10–500 mg L^{-1} NO_3^-, 1–80 mg L^{-1} NO_2^-, nitrate/nitrite 100, 5–100 mg L^{-1} NO_3^-, 0.5–50 mg L^{-1} NO_2^-, peroxide H_2O_2 25, 0.5–25 mg L^{-1}, peroxide H_2O_2 100, 0.5–25 mg L^{-1}; pH-Fix 2.0–9.0). The strips are evaluated using the QUANTOFIX Relax unit optical reader (from Macherey-Nagel, GmbH, Düren, Germany), which allows quantitative analysis with high accuracy. The Nitrate/nitrite strips were calibrated with $NaNO_2$ and NH_4NO_3 solutions of known concentrations, and the calibration was checked with UV–VIS absorption spectroscopy measurements. The use of strips allows the ageing of samples to be monitored with a time resolution of 1 min without significant consumption of the sample (i.e., the sample volume can be kept quasi-constant during ageing). The measurement error was set to less than 10%. The ageing dynamics of PAW depend on the pH, which was controlled by adding metal ion concentration to the water [26,27]. The Mg ions were added by inserting a solid piece of magnesium (5 g) immediately during the plasma treatment and left in the liquid for one hour after the treatment. The conductivity of PAW was measured after treatment and during ageing using the conductivity probe (Metrohm 914 pH/DO/Conductometer Pt 1000/B/O). After treatment with the plasma jet, the conductivity of the sample increases to about 27 µS/cm. Each treatment was monitored by optical emission spectroscopy (OES). The spectra show the presence of NO, N_2*. In addition, the concentrations of peroxide, nitrate/nitrite and pH were measured at 10 min intervals during treatment to ensure consistency of repeated treatment. For the March 2021 experiment, three PAW treatments were performed in volumes of 215 mL, mixed in the 2 L bottle and separated into 200 mL containers for storage and transport. From each treatment 15 mL of PAW was stored for ageing measurements over the next two weeks. PAW was characterized by the following physico-chemical elements, which are listed in Table 1.

Table 1. Physical-chemical characteristics of PAW.

Concentration of (in mg L^{-1})	H_2O_2	NO_2^-	NO_3^-	pH
40 min treatment (t_1, t_2, t_3)	4.1	3.3	11.2	6.1
	3.1	2.8	5.5	6.1
	0	1.4	5.4	5.8

2.2. Setting up the Experiment

The experiment was established at 9 March 2021 at the location Slobodnica (45°9′58″ N, 17°57′8″ E, an elevation of 87 m) in eastern Croatia in the trial filed and greenhouse of the University of Slavonski Brod. A three-factor trial was set up; seed varieties (V1, V2), growing conditions (greenhouse (G) and field (F)), and untreated and PAW treated seeds. Two seed varieties of crystal lettuce (Maradone (Lot: N87925, Vilmorin, France) and Minestrone (Lot: N78760, Vilmorin, France)) were obtained from commercial lettuce company. The PAW was prepared at the Institute of Physics in Zagreb and delivered to Slavonski Brod the day before lettuce sowing. A replicate of 4 × 50 seeds of each variety was soaked in PAW for one hour and then sown in modular trays (Pöpellmann TEKU (BP3153/60) 53 cm × 31 cm × 5.6 cm) with 60 sowing sites (volume of each site 76 mL) filled with cultivation medium Potgrond P (Klasmann-Deilmann). The other replicates of 4 × 50 seeds of each variety were soaked in untreated water and planted at the same time as a control batch. Greenhouse conditions were optimized to maximize seedling growth, with a pre-germination temperature of 15 °C and a post-germination temperature (10–12 °C during the day) and (6–10 °C during the night). After the seedlings reached the required growth stage (3–4 leaves) they were hardened off and planted in (Pöpellmann TEKU VTG 9)

9 cm × 6.8 cm (0.27 L) pots filled with ECO (Klasmann-Deilmann) batch cultivation media. The pots were tested under different growing conditions. One hundred pots of Maradone lettuce seeds treated with PAW were under greenhouse conditions and the other hundred pots were under field conditions. The control group was also grown under greenhouse and field conditions. The same was done with the Minestrone variety. The greenhouse was not specially heated or cooled, but was opened during the day and closed at night, as needed. Temperatures were measured daily and varied from 10 °C at night to 23 °C during the day. A total of 400 plants were grown in each experiment and 128 of them were randomly selected for measurements. The following biometric growth indicators were measured; rosette height, number of leaves, head mass, head diameter, root mass and root length. Plant mass was determined using an analytical balance with an accuracy of 0.01 g (PL3002, Mettler-Toledo International Inc., Greifensee, Switzerland Measurements were taken weekly on the following dates; 26 April 2021, 5 May 2021, 10 May 2021, 17 May 2021 and in the year 2022 at the following dates; 22 April 2022, 3 May 2022, 10 May 2022 and 17 May 2022 until lettuce reached commercial maturity. Each time biometric growth indicators of four plants of Maradona and Minestrone cultivars (per treatment and control) were measured in the greenhouse and in the field.

2.3. Dry Matter

Lettuce heads were measured four times and each time measured plants were dried at 60 °C for 3–4 days to the constant dry weight. The total dry weight of the plants was measured using an analytical balance an accuracy of 0.01 g (PL3002, Mettler-Toledo International Inc., Greifensee, Switzerland). Each time four plants of Maradona and Minestrone cultivars (per treatment and control) were measured in the greenhouse and in the field and the average dry weight of each plant was calculated by dividing the total dry weight by the number of plants. This was repeated 4 times and averaged again.

2.4. Statistical Analysis

The collected research data were statistically processed using the RStudio computer program, while the statistically significant differences (LSD) were calculated with the MS Excel program, 2019. According to Fisher's test on significance of variance analysis least significant differences (LSD) was calculated for $p < 0.05$ by comparing the means.

3. Results and Discussion

In the experiment, the influence of treating the seeds of two lettuce varieties with PAW on the development of the plants in greenhouse and in the field was studied. Each measured parameter (head mass, head diameter, root mass and length, number of leaves and rosette height) is presented in a separate table for 2021 and 2022. The measurement results are presented separately for the above-ground and below-ground parts of the plant, i.e., the root system.

3.1. Measurement of the Above-Ground Parts

The results of the measurement of the mass of the lettuce heads are shown in Table 2 for the year 2021 and in Table 3 for the year 2022.

In 2021 and 2022 (Tables 2 and 3), statistical significance was found between treatments C (Control) and PAW in the first measurement. In the other measurements there was no statistical difference between the treatments. In the second, third and fourth measurements, a statistically significant influence of growing conditions was found, and it was found that growing in a greenhouse gave a statistically better result.

The second parameter measured was the diameter of the lettuce head. The results are shown in Table 4 for the year 2021 and in Table 5 for the year 2022.

Table 2. The mass of the lettuce heads in the year 2021 (in g).

Sampling Date		Measurement 1st 26 April 2021		Measurement 2nd 3 May 2021		Measurement 3rd 10 May 2021		Measurement 4th 17 May 2021	
Variety	Growing Conditions	C	PAW	C	PAW	C	PAW	C	PAW
V1	Greenhouse	8.17Bb	13.65aB	36.3A	42.62A	96.96A	98.11B	197.29B	211.48B
V1	Field			25.77B	28.61B	74.63B	92.11B	152.34C	159.47C
V2	Greenhouse	12.52bA	17.61aA	35.63A	44.8A	95.65A	118.29A	234.76A	243.01A
V2	Field			18.39B	21.12B	80.91B	88.6B	195.02B	215.91B
F_V		* ($p < 0.05$, F = 15.52)		ns ($p < 0.05$, F = 1.15)		ns ($p < 0.05$, F = 0.74)		* ($p < 0.05$, F = 19.03)	
F_{GC}				* ($p < 0.05$, F = 27.57)		* ($p < 0.05$, F = 8.53)		* ($p < 0.05$, F = 18.05)	
F_{TS}		* ($p < 0.05$, F = 7.03)		ns ($p < 0.05$, F = 2.85)		ns ($p < 0.05$, F = 3.78)		ns ($p < 0.05$, F = 1.71)	

V1—variety 1; V2—variety 2; C—control; P—PAW; G—greenhouse; F—field; FV—F test lettuce variety; FGC—F test growing conditions; FBS—F test treatment before sowing; *—statistical significance; ns-no statistical significance; Values between the same line followed by different lowercase letters are statistically significantly different (LSD) at $p < 0.05$; Values within the same columns followed by different capital letters are statistically significantly different (LSD) at $p < 0.05$.

Table 3. The mass of the lettuce heads in the year 2022 (in g).

Sampling Date		Measurement 1st 22 April 2022		Measurement 2nd 3 May 2022		Measurement 3rd 10 May 2022		Measurement 4th 17 May 2022	
Variety	Growing Conditions	C	PAW	C	PAW	C	PAW	C	PAW
V1	Greenhouse	9.54bA	14.32aA	38.58A	48.01A	182.95A	201.18A	252.59B	305.57A
V1	Field			33.56B	43.17A	97.92B	74.36B	277.23B	267.72B
V2	Greenhouse	13.11bB	17.86aB	40.16A	46.08A	213.46A	188.14A	368.64A	283.88A
V2	Field			22.48B	35.52B	117.68B	105.16B	234.14B	227.28B
F_V		* ($p < 0.05$, F = 4.55)		ns ($p < 0.05$, F = 0.01)		ns ($p < 0.05$, F = 3.96)		ns ($p < 0.05$, F = 0.04)	
F_{GC}				* ($p < 0.05$, F = 33.67)		* ($p < 0.05$, F = 130.57)		* ($p < 0.05$, F = 13.49)	
F_{TS}		* ($p < 0.05$, F = 5.12)		ns ($p < 0.05$, F = 0.01)		ns ($p < 0.05$, F = 1.60)		ns ($p < 0.05$, F = 0.75)	

* Explanations: see Table 2.

Table 4. The diameter of the lettuce head in the year 2021 (in cm).

Sampling Date		Measurement 1st 26 April 2021		Measurement 2nd 3 May 2021		Measurement 3rd 10 May 2022		Measurement 4th 17 May 2022	
Variety	Growing Conditions	C	PAW	C	PAW	C	PAW	C	PAW
V1	Greenhouse	10.25B	10.5B	19AB	20A	25.13bA	28aA	28.25A	29.25A
V1	Field			18B	17.75B	22.25B	23.5B	23.5B	24.25B
V2	Greenhouse	12.13bA	15.25aA	19.75A	21.5A	27A	28A	27.25A	29.5A
V2	Field			15.75C	17.25B	22.25bB	25.25bB	26.25A	26.75B
F_V		* ($p < 0.05$, F = 65.64)		ns ($p < 0.05$, F = 0.02)		ns ($p < 0.05$, F = 1.02)		* ($p < 0.05$, F = 2.95)	
F_{GC}				* ($p < 0.05$, F = 11.8)		* ($p < 0.05$, F = 37.1)		* ($p < 0.05$, F = 26.51)	
F_{TS}		* ($p < 0.05$, F = 0.27)		ns ($p < 0.05$, F = 1.43)		* ($p < 0.05$, F = 7.48)		ns ($p < 0.05$, F = 2.95)	

* Explanations: see Table 2.

Table 5. The diameter of the lettuce head in the year 2022 (in cm).

Sampling Date		Measurement 1st 22 April 2022		Measurement 2nd 3 May 2022		Measurement 3rd 10 May 2022		Measurement 4th 17 May 2022	
Variety	Growing Conditions	C	PAW	C	PAW	C	PAW	C	PAW
V1	Greenhouse	9.2bB	14.18aB	20.5A	20.5A	29.75aA	27.75bA	24.25CD	29.25B
V1	Field			15.88B	162.5B	21.5B	22.5B	25.25C	23C
V2	Greenhouse	14.13bA	17.25aA	20A	20.25A	28aA	26.25bA	34A	34.25A
V2	Field			18C	20.75A	23.3aB	19.13bB	28.75B	24.25C
F_V		* ($p < 0.05$, F = 9.12)		ns ($p < 0.05$, F = 2.66)		ns ($p < 0.05$, F = 2.92)		* ($p < 0.05$, F = 19.97)	
F_{GC}				* ($p < 0.05$, F = 8.31)		* ($p < 0.05$, F = 76.04)		* ($p < 0.05$, F = 22.07)	
F_{TS}		* ($p < 0.05$, F = 8.28)		ns ($p < 0.05$, F = 0.88)		* ($p < 0.05$, F = 5.33)		ns ($p < 0.05$, F = 0.12)	

* Explanations: see Table 2.

In the first measurement of head diameter in the years 2021 and 2002 statistical significance was found for the following parameters: cultivar and seed treatment method. In the second, third and fourth measurement, a statistically significant difference was found in relation to growing conditions, and it was found that growing in a greenhouse gave a statistically better result.

The third parameter measured was the number of the leaves and results are shown in Table 6 for the year 2021 and in Table 7 for the year 2022.

Table 6. The number of the leaves in the year 2021.

Sampling Date		Measurement 1st 26 April 2021		Measurement 2nd 3 May 2021		Measurement 3rd 10 May 2021		Measurement 4th 17 May 2021	
Variety	Growing Conditions	C	PAW	C	PAW	C	PAW	C	PAW
V1	Greenhouse	9.50b	10.75a	16.5A	17.5A	23A	24.5A	36.25A	36.25A
V1	Field			15.5A	16.25A	20B	20B	30.25B	31C
V2	Greenhouse	8.5b	9.75a	13.25B	13.5B	20B	21.5C	30.5B	33.75B
V2	Field			11.75B	11.75B	18.25B	19B	26.5C	28.5D
F_V		ns ($p < 0.05$, F = 0.83)		* ($p < 0.05$, F = 33.1)		* ($p < 0.05$, F = 4.97)		* ($p < 0.05$, F = 12.01)	
F_{GC}				ns ($p < 0.05$, F = 4.17)		* ($p < 0.05$, F = 8.97)		* ($p < 0.05$, F = 24.01)	
F_{TS}		* ($p < 0.05$, F = 6.21)		ns ($p < 0.05$, F = 0.55)		ns ($p < 0.05$, F = 0.91)		ns ($p < 0.05$, F = 2.06)	

* Explanations: see Table 2.

Table 7. The number of the leaves in the year 2022.

Sampling Date		Measurement 1st 22 April 2022		Measurement 2nd 5 May 2022		Measurement 3rd 10 May 2022		Measurement 4th 17 May 2022	
Variety	Growing Conditions	C	PAW	C	PAW	C	PAW	C	PAW
V1	Greenhouse	8.75b	10.5a	16A	17A	24.5A	24.25A	27.25B	32.75B
V1	Field			17.75A	15.5AB	23.75A	25.25A	36.25A	36.5A
V2	Greenhouse	9.75b	11.25a	12B	14BC	19.75B	21.5B	29B	29.75BC
V2	Field			11B	11.5C	18.5B	20.75B	28.75B	27.5C
F_V		ns ($p < 0.05$, F = 3.39)		* ($p < 0.05$, F = 12.09)		* ($p < 0.05$, F = 17.44)		* ($p < 0.05$, F = 8.84)	
F_{GC}				ns ($p < 0.05$, F = 0.41)		ns ($p < 0.05$, F = 0.18)		ns ($p < 0.05$, F = 2.95)	
F_{TS}		* ($p < 0.05$, F = 7.89)		ns ($p < 0.05$, F = 0.06)		ns ($p < 0.05$, F = 1.62)		ns ($p < 0.05$, F = 0.77)	

* Explanations: see Table 2.

In 2021 and 2022, in the first measurement, seed treatment had statistically significant effect on the number of leaves. Growing conditions didn't have a significant effect, unlike the previous parameters. The LSD test showed statistical significance of the number of leaves between seed treatment C and PAW, which was the same for both cultivars (1.25 pcs).

The fourth parameter measured was the height of the rosette which is shown in Tables 8 and 9.

Table 8. The height of the rosette in the year 2021 (in cm).

Sampling Date		**Measurement 1st 26 April 2021**		**Measurement 2nd 3 May 2021**		**Measurement 3rd 10 May 2021**		**Measurement 4th 17 May 2021**	
Variety	**Growing Conditions**	**C**	**PAW**	**C**	**PAW**	**C**	**PAW**	**C**	**PAW**
V1	Greenhouse	6.75bB	9.5aB	11A	10.5A	13.5B	15.25A	16.25A	16.75A
V1	Field			8.75B	9.5A	11.63C	12.25C	13.25D	13.63C
V2	Greenhouse	10.63A	10.88A	10.5A	11.75A	15.25A	15.75A	15.38bB	17aA
V2	Field			8.75B	8B	12.25C	13C	14.25C	14.88B
F_V		* ($p < 0.05$, F = 23.42)		ns ($p < 0.05$, F = 0.87)		* ($p < 0.05$, F = 6.26)		ns ($p < 0.05$, F = 1.24)	
F_{GC}				* ($p < 0.05$, F = 16.0)		* ($p < 0.05$, F = 31.9)		*($p < 0.05$, F = 41.3)	
F_{TS}		* ($p < 0.05$, F = 7.65)		ns ($p < 0.05$, F = 0.12)		ns ($p < 0.05$, F = 2.7)		* ($p < 0.05$, F = 4.58)	

* Explanations: see Table 2.

Table 9. The height of the rosette in the year 2022 (in cm).

Sampling Date		**Measurement 1st 22 April 2022**		**Measurement 2nd 5 May 2022**		**Measurement 3rd 10 May 2022**		**Measurement 4th 17 May 2022**	
Variety	**Growing Conditions**	**C**	**PAW**	**C**	**PAW**	**C**	**PAW**	**C**	**PAW**
V1	Greenhouse	6.5bB	9.7aB	11.5A	11.38A	14.13A	16.75A	16.63aA	14.13b
V1	Field			10B	9.63B	10.88C	10.13C	14.5aB	13b
V2	Greenhouse	7.38bA	10.88aA	9.63B	11.5A	12B	11.75B	17.13aA	13.75b
V2	Field			9.5B	9.63B	7.75D	8.88D	11.63bC	13.25a
F_V		* ($p < 0.05$, F = 7.27)		ns ($p < 0.05$, F = 0,87)		* ($p < 0.05$, F = 30.82)		ns ($p < 0.05$, F = 0.89)	
F_{GC}				* ($p < 0.05$, F = 4.74)		* ($p < 0.05$, F = 67.34)		*($p < 0.05$, F = 12.19)	
F_{TS}		* ($p < 0.05$, F = 13.85)		ns ($p < 0.05$, F = 0.39)		ns ($p < 0.05$, F = 1.76)		* ($p < 0.05$, F = 4.71)	

* Explanations: see Table 2.

Tables 8 and 9 show that rosette height of was significantly affected by the cultivar and seed treatment in the first measurement. In the other measurements the rosette height was significantly influenced by the growing conditions and it was found that growing in the greenhouse gave a statistically better result.

3.2. Measurement of the Root Parameters

In this part, the results of the measurement of the below-ground part of the plants are presented. The first parameter measured was root mass which is presented in Table 10 for the year 2021 and in Table 11 for the year 2022.

As can be seen, root mass was statistically significantly affected by cultivar in 2021 and 2022. The influence of seed treatment with PAW was absent. With the same cultivar, there was no statistically significant difference between treatment C and PAW.

Table 10. The root mass in the year 2021 (in g).

Sampling Date		Measurement 1st 26 April 2021		Measurement 2nd 3 May 2021		Measurement 3rd 10 May 2021	
Variety	**Growing Conditions**	**C**	**PAW**	**C**	**PAW**	**C**	**PAW**
V1	Greenhouse	8.76A	9.08A	36.31	37.96	31.56	37.30
V1	Field			18.52	20.28	27.29	28.32
V2	Greenhouse	4.02B	3.65B	13.82	23.46	27.34	30.40
V2	Field			12.73	13.73	16.16	25.23
F_V		* ($p < 0.05$, F = 9.26)		ns ($p < 0.05$, F = 3.34)		ns ($p < 0.05$, F = 1.74)	
F_{GC}				ns ($p < 0.05$, F = 2.94)		ns ($p < 0.05$, F = 2.38)	
F_{TS}		ns ($p < 0.05$, F = 0.0)		ns ($p < 0.05$, F = 0.27)		ns ($p < 0.05$, F = 0.97)	

* Explanations: see Table 2.

Table 11. The root mass in the year 2022 (in g).

Sampling Date		Measurement 1st 22 April 2022		Measurement 2nd 3 May 2022		Measurement 3rd 10 May 2022	
Variety	**Growing Conditions**	**C**	**PAW**	**C**	**PAW**	**C**	**PAW**
V1	Greenhouse	8.82A	9.12 A	37.24	26.75	38.45	37.96
V1	Field			18.89	20.54	27.48	28.51
V2	Greenhouse	3.82B	3.47B	17.58	22.56	26.84	29.56
V2	Field			12.82	14.52	17.12	15.16
F_V		* ($p < 0.05$, F = 10.13)		ns ($p < 0.05$, F = 4.01)		ns ($p < 0.05$, F = 1.03)	
F_{GC}				ns ($p < 0.05$, F = 3.25)		ns ($p < 0.05$, F = 3.18)	
F_{TS}		ns ($p < 0.05$, F = 0.2)		ns ($p < 0.05$, F = 0.98)		ns ($p < 0.05$, F = 1.84)	

* Explanations: see Table 2.

The second parameter measured was the root length and the measurement results are shown in Tables 12 and 13.

Table 12. The root length in the year 2021 (in cm).

Sampling Date		Measurement 1st 26 April 2021		Measurement 2nd 3 May 2021		Measurement 3rd 10 May 2021	
Variety	**Growing Conditions**	**C**	**PAW**	**C**	**PAW**	**C**	**PAW**
V1	Greenhouse	19.25B	20.00	26.5A	26.75A	25A	26.75A
V1	Field			15.25B	16.B	16.25bB	24.5aA
V2	Greenhouse	24A	24.00	23.25A	235A	2.45bA	29aA
V2	Field			22A	22.5A	22A	22B
F_V		* ($p < 0.05$, F = 4.85)		ns ($p < 0.05$, F = 0.55)		ns ($p < 0.05$, F = 0.69)	
F_{GC}				* ($p < 0.05$, F = 7.2)		* ($p < 0.05$, F = 1.01)	
F_{TS}		ns ($p < 0.05$, F = 0.04)		ns ($p < 0.05$, F = 0.07)		* ($p < 0.05$, F = 4.48)	

* Explanations: see Table 2.

Table 13. The root length in the year 2022 (in cm).

Sampling Date		Measurement 1st 22 April 2022		Measurement 2nd 3 May 2022		Measurement 3rd 10 May 2022	
Variety	**Growing Conditions**	**C**	**PAW**	**C**	**PAW**	**C**	**PAW**
V1	Greenhouse	20.51B	21.2	26.7A	27.08A	26.89A	26.75A
V1	Field			15.85B	16.88B	19.15B	23.8A
V2	Greenhouse	24.36A	24.45	23.78A	24.89A	26.98A	29.18B
V2	Field			22.15A	23.01A	22.13AB	22.46A
F_V		* ($p < 0.05$, F = 4.26)		ns ($p < 0.05$, F = 1.63)		ns ($p < 0.05$, F = 3.02)	
F_{GC}				* ($p < 0.05$, F = 8.84)		* ($p < 0.05$, F = 4.75)	
F_{TS}		ns ($p < 0.05$, F = 0.47)		ns ($p < 0.05$, F = 0.12)		ns ($p < 0.05$, F = 2.32)	

* Explanations: see Table 2.

3.3. Dry Matter

The next parameter determined was dry matter (Figure 1 for the year 2021 and Figure 2 for the year 2022).

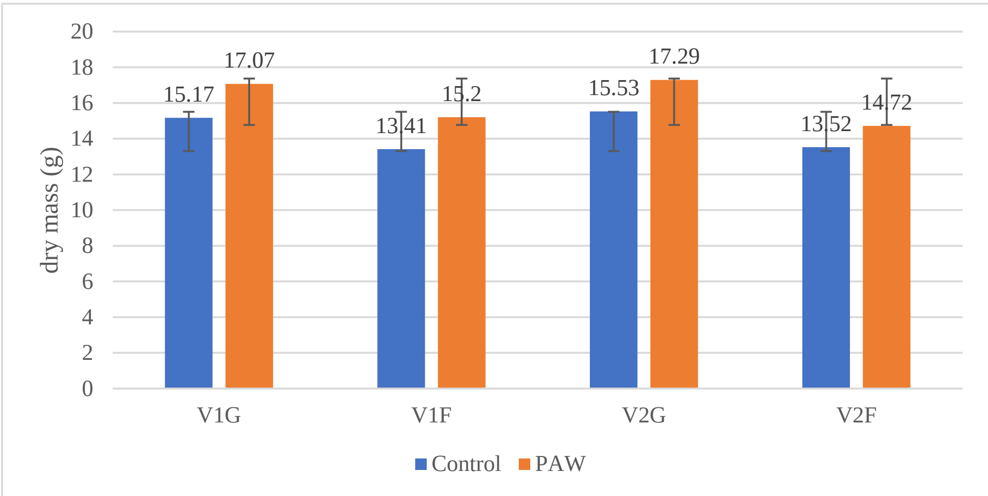

Figure 1. Dry matter content of lettuce head in 2021.

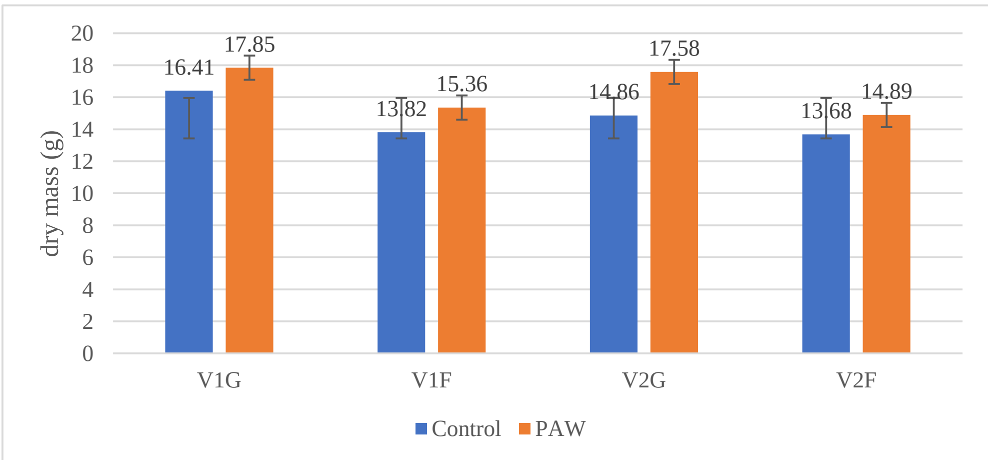

Figure 2. Dry matter content of lettuce head in 2022.

As shown in Figure 1 for 2021, the average dry weight ranged from 13.68 g (lowest value) to 17.85 g (highest value), and no statistically significant influence was found between measurements ($p = 0.2296$).

Figure 2 for the year 2022 shows that the mass of dry matter ranged from 13.82 g as the lowest value to 17.85 g as the highest value and no statistical difference was found ($p = 0.5398$). As can be seen in Figures 1 and 2, the PAW treatment had a positive impact on the dry matter of the measured plants. Figure 1 shows that V1PG had a dry matter 12.52% higher than V1CG and V1PF had a dry matter 13.34% higher than V1CF. V2PG had a dry matter 11.33% higher than V2CG. V2PF had a dry matter 8.87% higher than V2CF. Figure shows that V1PG had a dry matter 8.07% higher than V1CG and V1PF had a dry matter 11.14% higher than V1CF. V2PG had a dry matter 18.3% higher than V2CG. Also, V2PF had a dry matter 8.84% higher than V2CF.

4. Discussion

PAW is used in agriculture to increase protection against biotic and abiotic stress. It is environmentally friendly and can be used in organic farming or as an alternative to chemical biostimulants and artificial fertilizers. In addition, PAW affects plants in various ways; protein synthesis, gene expression, enzyme activity, DNA methylation, DNA demethylation, DNA damage, morphological and chemical changes in seed coat, plant hormonal balance, germination and seedling growth. In the conducted research, we tried to find out the influence of soaking of lettuce seeds in the PAW on further development of plants. According to Holubova et al. [28], there are many studies in which a positive influence of PAW on germination and development of plants was found, and many in which there were no results or they were negative. However, an optimal dose of PAW cannot be generally stated because different plant species respond differently to treatment with PAW. Therefore, we tried to find out how long the seeds must be soaked in PAW to obtain good results, because according to Filatova et al. [29] too short an exposure time has no effect, while too long exposure time can have a detrimental effect. From the obtained results it can be concluded that the seed treatment had an influence on the development of the above-ground part of the plants in the first stage of development, which can be seen from the Tables 2–9. Similar results were obtained by Sarinont et al. [30] who found that the average plant length was higher in plasma-irradiated radish seeds than in the control samples. Dobrin et al. [31] also obtained similar results when they studied wheat. Puač et al. [32] and Sivachandiran et al. [33] also found that the treated plants had better germination, improved enzyme activity and better developed plants. The tables show a statistically significant influence of PAW in the first measurement of the following parameters: mass of the head, number of leaves and height of the rosette, indicating the possibility that PAW treatment improves the growth of early seedling. Comparable results were obtained by Abbaszadeh et al. [34] and Kučerova et al. [35] who reported that PAW treatment on seeds improved the growth of early seedlings. Kučerova et al. [35] found that seeds cultivated at PAW interacted mainly with H_2O_2 at the early growth stages during imbibition and germination, while NO_2^- and NO_3^- were metabolized after the onset of germination and during early plant growth which also confirmed the assumption that the seedlings were still under the influence of PAW treatment in first measurement. From the presented research results, it is also evident that cultivation in a protected area led to significantly better results in seedling growth in terms of lettuce head, which is consistent with the results of Kang et al. [36] whose results indicated that the improvement in plant growth and stress tolerance could be explained by cultivation in greenhouse. The growing conditions in the protected area were not specifically adapted; there was no cooling of the area. The greenhouse was opened in the morning and closed in the evening. Nevertheless, the plants grown in the greenhouse were significantly larger and of better quality than those grown in the field. The increased CO_2 concentration in the air at night and shading during the day probably contributed to this development. Pearson et al. [37] found that an increase in CO_2 concentration resulted in an increase in final head weight of lettuce.

According to Sarinont et al. [30] the growth promoting effects become weaker with time and this could be an explanation for the lack of effect on subsequent development. As for the root part, no positive result was found in the first measurement. Similar results were also obtained by others [30,33,34]. According to Guragin et al. [38] no significant increase in root length was observed in PAW and deionized irrigated radish and pea seedlings. This can be explained by the fact that PAW is rich in nitrogen and not in phosphorus, which is essential for root development. As for dry matter, it was found that the plants under PAW had higher dry matter than the untreated plants. Similar results were obtained by Ling et al. [39] who observed an increase in the shoot and root dry weight (21.95% and 27.51%, respectively) in soybean compared to untreated plants (control). According to Stoleru et al. [40] who found similar results on lettuce this mass could be explained by a stimulation of protein synthesis, induced by PAW. A similar conclusion was reached by Takaki et al. [41] in studying of *Brassica rapa* and Matra [42], who found that plasma treatment increased the dry matter of radish by 9–12%.

The results are also comparable to recent findings by Japundžić-Palenkić et al. [43] but research should certainly be expanded to obtain more detailed information on this topic.

5. Conclusions

According to the research results, it can be concluded that cultivation under greenhouse conditions gave better results for all measured morphological characteristics: head mass, rosette length, number of leaves, rosette diameter, root length and root mass.

Seed treatment with PAW had a positive effect on seedlings growth in the first stage of development only for the above-ground parts (head mass, rosette length, number of leaves, rosette diameter), in the first measurement compared to the control, but in the following measurements this effect was lost. In addition, there was no influence on the below-ground parts (root mass and length).

Lettuce grown from seeds treated with PAW had a higher percentage of dry matter than the control plants.

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