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Gliding Arc Plasma Treatment of Maize (*Zea mays* L.) Grains Promotes Seed Germination and Early Growth, Affecting Hormone Pools, but Not Significantly Photosynthetic Parameters

Božena Šerá [1,*], Radomíra Vanková [2], Karel Roháček [3] and Michal Šerý [4]

[1] Department of Environmental Ecology and Landscape Management, Faculty of Natural Sciences, Comenius University in Bratislava, Ilkovičova 6, 842 15 Bratislava, Slovakia
[2] Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany of the Czech Academy of Sciences, Rozvojová 263, 165 02 Prague, Czech Republic; vankova@ueb.cas.cz
[3] Faculty of Health and Social Sciences, Institute of Laboratory Diagnostics and Public Health, University of South Bohemia, Boreckého 1167/27, 370 11 Ceske Budejovice, Czech Republic; krohacek@zsf.jcu.cz
[4] Department of Physics, Faculty of Education, University of South Bohemia, Jeronýmova 10, 371 15 Ceske Budejovice, Czech Republic; kyklop@pf.jcu.cz
* Correspondence: bozena.sera@uniba.sk

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Abstract: Maize grains (*Zea mays* convar. *Indentata* Sturt.) were treated with non-thermal plasma, where Gliding Arc plasma discharge at an atmospheric pressure was used (working gas: Air; time duration: 0 s, 180 s, 300 s, 600 s). The experiment was conducted at a temperature of 18 °C, light/dark 12/12 h, and a light intensity of 100 µmol/m^{2}s. Seed germination, seedling growth, photosynthetic parameters, and hormone (abscisic acid, jasmonic acid, salicylic acid, indole-3-acetic acid, and cytokinin) contents were measured. The highest stimulation of seed germination (to 141%), root length (to 221%), shoot length (to 298%), and root weight (to 122%) in comparison with the control was recorded after Gliding Arc plasma treatment for 600 s. The photochemical and non-photochemical Chl fluorescence parameters were not significantly affected by Gliding Arc plasma treatment. In contrast, hormonal pools in maize were significantly affected. The short-term plasma treatment (180 s) was associated with a decrease in the stress hormones abscisic acid, salicylic acid, jasmonic acid, and jasmonate isoleucine, while indole-3-acetic acid and cytokinin precursors were elevated. Longer-term treatment (300 s, 600 s) had an opposite effect—an elevation of abscisic acid, jasmonic acid, and jasmonate isoleucine as well as active cytokinins. The content of auxin decreased. Gliding plasma treatment may significantly affect maize physiology, dependent on the treatment duration.

Keywords: cold plasma; non-thermal plasma; seed stimulation; phytohormones; photosynthetic parameters

1. Introduction

Non-thermal plasma (NTP) can promote chemical reactions at a lower operating temperature range and can be used in various biological, medicinal, agronomical, food, and forestry applications [1]. NTP belongs to alternative (unconventional) biotechnological methods in plant agriculture and the food industry [2,3]. Unconventional approaches may contribute to the reduction of the burden on the environment and the increase in food quality and safety.

Plasma is a charged gas with strong electrostatic interactions consisting of neutral and excited atoms, free radicals, negative and positive ions, and UV photons with a net electric charge of zero [4]. As NTP contains low-temperature particles such as neutral molecules, atomic species, and relatively high-temperature electrons, it is cold and does not affect sensitive materials when comes into contact with them [4–6].

NTP treatment has the ability to prompt the seed germination rate, decrease surface infection, and impede the growth of pathogens. Seed germination is a deciding factor for seedling development in field conditions, which is influenced by water imbibition of the seed coat. Numerous physiological and biochemical processes such as protein synthesis, enzymatic stimulation, and starch digestion are involved in seed germination activity. Seed treatment with specific plasma triggers specific biochemical changes and also induces positive physical changes in the seed, ultimately enhancing the germination rate.

The use of NTP in agriculture, the food industry, and plant physiology has been reported in recent years [2,3,7–10]. The stimulation of plants with NTP treatment to promote seed germination and increase yield quantities and quality has attracted interest worldwide. Current papers show that cold plasma has a positive effect on seed germination and early seedling growth of wheat [9,11,12], rice [13], soybean [14,15], oilseed rape [16], and other agronomically and economically important crops. Nevertheless, it has been found that germination and initial growth depend on the time of exposure, the type of plasma used [17,18], and also the type of plant species [19,20].

Maize (*Zea mays* L.) is a monocotyledonous species (Poaceae family) and is one of the most-produced crops around the world. Improving the existing agricultural technologies and introducing new ones can further promote the cultivation of this crop, especially in the context of ongoing climate change. Only a few experiments in maize have been focused on testing the effect of NTP treatment. The aim of this paper is the elucidation of the effect of non-thermal plasma generated at atmospheric pressure on the initial development and growth of maize plants with a focus on selected physiological parameters associated with seed germination, photosynthetic processes, and phytohormonal activity of young plants. The results may serve as a guide for the further development of this biotechnological method of treatment of agriculturally important seeds and fruits. Maize was chosen as the test species due to its importance as a worldwide-spread crop.

2. Materials and Methods

2.1. NTP Treatment

Maize grains (*Zea mays* convar. *Indentata* Sturt.) were treated with a Gliding Arc sliding arc discharge. The Gliding Arc apparatus consisted of two 2-mm-thick aluminum electrodes, connected via an AUPEM converter and powered by the mains. Maize grains were placed 250 mm below the electrodes to minimize thermal effects (a maximum temperature of 50 °C). The working gas was steam water passing from the compressor through a deionized water flask with a flow rate of 10 L·min^{-1}. A schematic representation of the apparatus is shown in Figure 1, and a more detailed description can be found in Gavril et al. [21].

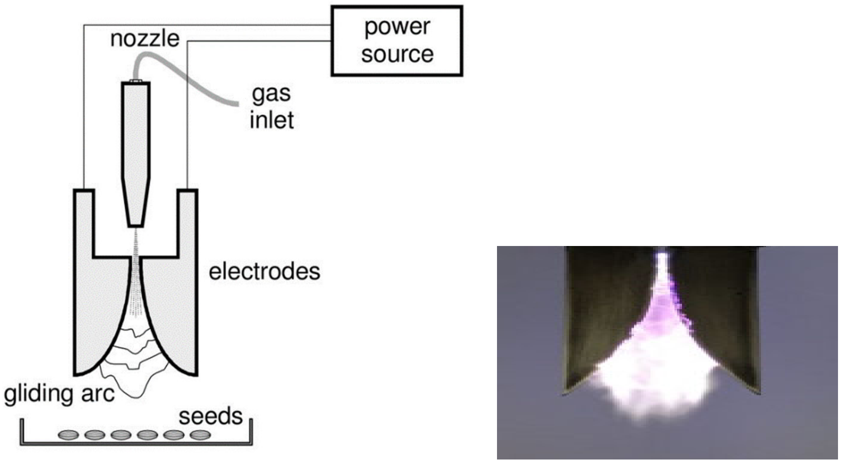

Figure 1. Scheme of Gliding Arc apparatus and detail of Gliding Arc plasma discharge between electrodes. Reprinted with permission from Šerá et al. [20] (2017/Springer).

The duration of the plasma treatments was 0 s (control set), 180 s, 300 s, and 600 s; 150 grains were used for each treatment.

2.2. Seed Germination and Early Growth

The experiment consisting of four experimental variants was conducted in five replications. Each replicate containing 30 grains was placed into Petri dishes (90 mm in diameter) with four layers of filter paper (KA 0/80) and 6 mL of deionized water. All grains were incubated in the dark at a temperature ca 20 °C for one week.

The number of germinated seeds, length of roots and shoots were measured on the 4th and 6th days from the first seed germination occurrence; the fresh weight of roots and shoots was measured on the 6th day. Germinated seeds were considered those with at least a 1 mm long radicle protrusion. Seed germination was calculated by the formula:

$$\mathrm{SG}\ (\%) = \frac{\sum_{i=1}^{5} \frac{\mathrm{N}_{P_i}(j)}{30}}{5} \times 100, \tag{1}$$

where SG is seed germination, i is the index of the petri dish, j is the index of the cultivation day, and $\mathrm{N}_{\mathrm{P}i}(j)$ is the number of germinated seeds per i-petri dish in j day.

Collected roots and shoots were weighed on a laboratory scale (Sartorius, balance sensitivity 0.001 g). Seed germination and characteristics of early growth were measured according to Šerá et al. [22].

2.3. Plant Cultivation

Plastic containers (30 × 38 × 25 cm) with a substrate volume ca 11.5 L were used in the plant cultivation. The substrate used was 'Horticultural substrate B universal' (fa Rašelina, Soběslav, Czech Republic), which was sieved through a sieve of 3 × 3 mm. Each experimental variant was cultivated in four containers, which represented the replications. In each container, 30 seeds were placed in regular rows, each to a depth of 2 cm. The filled containers were placed in a culture room. Air temperature and humidity were monitored during plant cultivation ('Digital Humidity/Temperature Meter', CH.BEHA GmbH, Glottertal, Germany), and the average values were 23.5 ± 1.0 °C and 42.9 ± 4.8%, respectively.

Soil moisture was adjusted to about 30% (measured with the HH2 Moisture Meter and probe WET Sensor type WET-2, fa Delta-T Devices Ltd., Cambridge, UK) and maintained at this value. The mean value of the irradiance (PFD—Photon Flux Density) at the level of the upper edge of containers with artificial light from fluorescent lamps (light blue–white and light yellow–white light) was 37.8 ± 10.9 $\mu\mathrm{mol}_{\mathrm{photons}} \cdot \mathrm{m}^{-2} \cdot \mathrm{s}^{-1}$. The following light regime was maintained: 12 h day (7–19) and 12 h dark (19–7).

After 14 days, plants (12 individuals per one treatment) were harvested and frozen at −80°C for hormonal analysis. Then, 25-day-old plants (10 individuals per one treatment) were subjected to determination of the photosynthetic parameters.

2.4. Photosynthetic Activity

Photosynthetic activity of maize plants was evaluated in 25-day-old plants, being measured for the next 7 days during the light period. In each container (four replicates of each treatment), 10 chlorophyll fluorescence measurements were performed on central parts of well-developed 1–2 leaves of 5–7 plants placed considerately within a ventilated leaf holder. The leaves of approximately the same age were selected and some plants were repeatedly used for fluorescence measurements during these 7 days.

The parameters of the efficiency of photosynthetic processes of both photochemical and non-photochemical natures were determined from the slow Chl *a* fluorescence induction kinetic curves recorded in a non-invasive manner with the PAM-2000 device (H. Walz, Effeltrich, Germany) according to Roháček [23]. The curves were measured at the following settings: Measuring radiation λ 650 nm, PFD $\leq 0.1\ \mu\mathrm{mol}_{\mathrm{fotons}} \cdot \mathrm{m}^{-2} \cdot \mathrm{s}^{-1}$, PAM-frequency of 600 Hz, actinic radiation λ 655 nm, PAM-frequency of 20 kHz, PFD of

40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (adapted to irradiance in the plant culture), saturation pulses with PFD ≈ 10^3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (white light), and FR radiation at λ 735 nm, PFD ≈ 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Fluorescence parameter values were evaluated according to Roháček [24] for the following photochemical (q_P, Φ_{Po}, Φ_2) and non-photochemical (q_E, q_N, NPQ) parameters: q_P reflects the actual fraction of open (re-oxidized) reaction centers of photosystem II (PS2), Φ_{Po} determines the maximum photochemical capacity of PS2, Φ_2 reflects the actual efficiency of photochemical conversion of radiant energy in PS2 during photosynthesis, and NPQ, q_N, and q_E quantify the efficiency of non-photochemical processes in the light-adapted states, such as pH-gradient formation (q_E), activation of a number of regulatory mechanisms (q_N), and dissipation of excitation energy to heat (NPQ).

2.5. Phytohormone Analysis

Maize grains treated with non-thermal plasma (12 grains per treatment) were grown to the age of 14 days (first leaves developed). Leaf samples (ca. 50 mg FW) were taken from the plants and frozen in liquid nitrogen. The samples were homogenized and extracted with methanol/water/formic acid (15/4/1, *v/v/v*, −20 °C) according to Dobrev and Kamínek [25] and Dobrev and Vaňková [26]. The labelled internal standards (10 pmol/sample) were added: $^{13}C_6$-IAA (Cambridge Isotope); $^2H_4$-SA (Sigma—Aldrich); $^2H_3$-PA, $^2H_3$-DPA (NRC—PBI); $^2H_6$-ABA, $^2H_5$-JA, $^2H_5$-transZ, $^2H_5$-transZR, $^2H_5$-transZ7G, $^2H_5$-transZ9G, $^2H_5$-transZOG, $^2H_5$-transZROG, $^2H_5$-transZRMP, $^2H_3$-DZ, $^2H_3$-DZR, $^2H_3$-DZ9G, $^2H_6$-iP, $^2H_6$-iPR, $^2H_6$-iP7G, $^2H_6$-iP9G, $^2H_6$-iPRMP (Olchemim).

The phytohormones were separated on a reverse-phase ion-exchange column (Oasis-MCX). Acid hormones (auxins, ABA, SA, JA) were eluted with methanol and basic hormones (cytokinins) were eluted with 0.35 M NH4OH in 60% methanol. Fractions were analyzed by HPLC (Ultimate 3000, Dionex) in combination with a mass spectrometer (3200 Q TRAP hybrid triple quadrupole with linear ion trap, Applied Biosystems).

2.6. Data Analysis

The obtained data were analyzed after logarithmic transformation using standard statistical tests determining the difference of variants (one-way ANOVA, Tukey HSD test, $\alpha < 0.05$). The statistical significance of the data on the photosynthetic parameters was tested by a paired *t*-test.

3. Results

3.1. Seed Germination and Early Growth

Seed germination and initial seedling growth parameters showed statistically significant effects of plasma treatments (Table 1). Gliding Arc plasma treatments for 180 s, 300 s, and 600 s exhibited positive effects on seed germination, which increased on the fourth day by 19%, 31%, and 41%, respectively, in relation to the control. On the sixth day, no significant effect on seed germination was observed.

All NTP treatments positively affected the root and shoot lengths. No significant differences among them were observed (Table 1). Root and shoot lengths were elevated most after Gliding Arc plasma treatment with a duration of 600 s; the lengths of the roots on the fourth and sixth days increased by 121% and 30%, respectively, while the lengths of the shoots on the fourth and sixth days increased by 198% and 26%, respectively, compared to the control. These monitored parameters revealed a significant difference between treatments and the control (Table 1).

The highest values of fresh root and shoot weights were recorded for Gliding Arc plasma treatment for 600 s, where fresh root weight increased by 22% and fresh shoot weight by 21% in relation to the control (Table 1). The statistical analysis showed the positive effect of the treatments on fresh root weight, while in the case of shoots, the difference did not reach statistical significance.

Table 1. Basic characteristics of seed germination and seedling early growth in maize after Gliding Arc plasma treatment. Mean, standard deviation (SD), percentage relative to control (%), and results of Tukey HSD test for multiple comparisons (HSD) are presented. HSD—different letters indicate a significant statistical difference $p < 0.05$.

Treatment	Seed Germination 4th Day (%)				Seed Germination 6th Day (%)				Root Length 4th Day (mm)				Shoot Length 4th Day (mm)			
	Mean	SD	%	HSD	Mean	SD	%	HSD	Mean	SD	%	HSD	Mean	SD	%	HSD
Control	63.3	1.8	100.0	a	99.3	0.4	100.0	a	3.5	1.0	100.0	a	0.5	0.3	100.0	a
180 s	75.3	4.1	122.1	b	100.0	0.0	100.7	a	5.1	1.5	147.1	b	0.7	0.1	151.9	b
300 s	82.6	3.3	130.5	b	99.3	0.4	100.0	a	5.3	0.8	151.5	b	0.8	0.4	160.3	b
600 s	89.3	4.1	141.1	b	100.0	0.0	100.7	a	7.7	1.3	221.3	b	1.4	0.5	298.3	b

Treatment	Root Length 6th Day (mm)				Shoot Length 6th Day (mm)				Fresh Root Weight (g)				Fresh Shoot Weight (g)			
	Mean	SD	%	HSD	Mean	SD	%	HSD	Mean	SD	%	HSD	Mean	SD	%	HSD
Control	31.3	1.8	100.0	a	13.1	0.8	100.0	a	180.1	18.3	100.0	a	995.0	116.9	100.0	a
180 s	38.2	6.0	122.1	b	15.4	1.3	117.7	a	198.9	19.2	110.5	b	1081.7	62.2	108.7	a
300 s	39.6	8.1	126.3	ab	15.3	2.7	117.0	a	206.5	22.1	114.7	b	1085.8	118.1	109.1	a
600 s	40.7	1.8	141.1	b	16.5	3.8	126.2	a	220.2	38.2	122.3	b	1200.7	117.7	120.7	a

3.2. Photosynthetic Activity

Gliding Arc plasma treatment had no significant effect on photosynthetic activities ($p \leq 0.05$); however, both positive and negative trends in the effect of plasma discharge on photosynthetic parameters were found. Generally, the positive effects of an NTP treatment on photosynthetic processes are represented by an increase in values of parameters of a photochemical nature (q_P, Φ_2) and, conversely, by a decrease in values of the non-photochemical parameters (NPQ, q_N, q_E). In the case of negative effects, the opposite is true. Thus, the positive effect compared to the control was found after 300 s of NTP treatment, while the negative effect was found after 600 s and especially after the 180 s treatment. The differences among the treatments, however, were not statistically significant (Figure 2). The almost constant values of the maximum photochemical capacity of PS2 (Φ_{Po} in Figure 2) for all treatments show that this parameter was not affected by the duration of plasma carcass exposure. Hence, the maximum photochemical capacity of PS2 centers within plant chloroplasts was maintained.

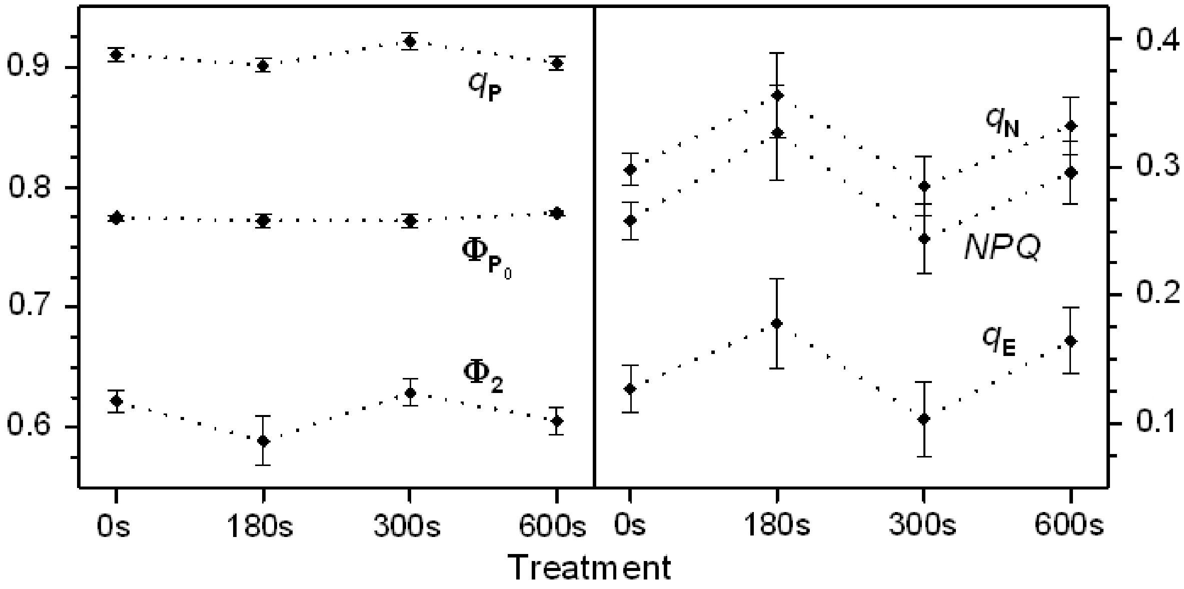

Figure 2. The photochemical (**left**) and non-photochemical (**right**) parameters evaluated on young maize plants dependent on the duration of the NTP treatment: q_P—the actual fraction of open reaction centers of PS2, Φ_{Po}—the maximum photochemical capacity of PS2, Φ_2—the actual efficiency of photochemical conversion of radiant energy in PS2, q_N—the non-photochemical quenching of variable fluorescence, NPQ—the non-photochemical quenching linked to dissipation of excitation energy to heat, q_E—the efficiency of pH-gradient formation. Trends shown in dotted lines.

3.3. Phytohormone Analysis

The phytohormone levels are presented in Table 2. Abscisic acid, the key hormone in response to abiotic stresses, showed a significant decrease after short-term treatment (by 60%), while after the 300-s treatment, an increase of 86% was observed. The levels of another stress hormone, jasmonic acid, decreased by 65% after the 180 s treatment, increasing the most after the 600 s treatment (44% compared to the control). Salicylic acid levels decreased by ca 35% after short-term treatment, increasing to control levels after t300 s. After NTP treatment for 600 s, a decrease of ca 21% was found. The levels of active cytokinins (trans-zeatin, isopentenyladenine, and cis-zeatin) slightly decreased after the 180 s plasma treatment, increasing moderately (by 24–29%) after longer-term treatments. The levels of their precursors (cytokinin phosphates) increased after all plasma treatments (by 190%, 190%, and 130%, after 180 s, 300 s, and 600 s respectively), which correlated well with the promoted seedling growth. In contrast, the levels of the most active auxin, indole-3-acetic acid, increased after short-term treatment (by 40% in comparison with the control), slightly decreasing after longer-term ones (by 12–18%).

Table 2. The impact of Gliding Arc plasma treatment on hormonal levels. ABA—abscisic acid, JA—jasmonic acid, JA-Ile—jasmonate isoleucine, SA—salicylic acid, IAA—indole-3-acetic acid, active CKs—active cytokinins (trans-zeatin + isopentenyladenine + cis/zeatin). Mean and standard deviation (SD) are presented.

Treatment	**ABA**		**JA**		**JA-Ile**		**SA**		**IAA**		**Active CKs**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Control	24.96	0.89	47.56	1.09	10.45	0.05	103.93	13.39	94.50	3.53	7.41	1.00
180 s	9.82	0.76	16.35	1.10	0.90	0.03	68.03	11.54	132.34	13.99	6.00	0.42
300 s	46.35	3.05	52.51	7.08	12.81	1.89	106.01	5.69	77.65	3.42	9.54	1.18
600 s	35.55	3.07	68.57	3.23	20.02	1.78	82.44	0.41	83.35	5.08	9.18	1.89

4. Discussion

The stimulation of maize seed germination is in accordance with the results of previous experiments [18,27–30], where the effects of NTP were recorded mainly in the initial stages of seed germination and initial seedling growth. Karmar et al. [31] reported that Low-Frequency Glow Discharge (mixed gas: Ar + O_2) plasma treatment of maize grains enhanced their germination rate by 15.88%, shoot length by 33.42%, and root length by 10.67% in respect to the controls. The NTP treatment durations were 30 s, 60 s, 90 s, and 120 s. In our experiment, another type of NTP apparatus (Gliding Arc apparatus) and working gas (air) were used. The most effective time was 600 s—seed germination increased by 41%, root length by 121%, shoot length by 198%, and root weight by 22% in relation to control samples (Table 1). The differences between the studies may be caused by the different maize cultivars used, the different type of working gas, the nature of the instruments used, and the type of plasma discharge.

Studies of Šerá et al. [17] and Ahn et al. [18] showed that different types of plasma apparatus are an important factor for seed response. It has also been found that seeds/fruits of different plant species respond to NTP treatment in different ways, where some plants are stimulated while others are inhibited. The difference in the response to NTP was found even in different cultivars of poppy (*Papaver somniferum* L.) and hemp (*Cannabis sativa* L.) [19,20]. Due to the great variability in methods of plasma generation, it is difficult to perform an overarching comparative study. Nevertheless, several studies have summarized these research topics, e.g., [2,8–10,20,32].

Gliding Arc plasma treatment of maize grains caused certain effects on the photochemical and non-photochemical processes in young maize leaves. Certain positive trends were evident (Figure 2) for the 300 s treatment, while negative trends were found mainly for the 180 s treatment. Some discrepancy between treatments may be due to differences in the leaf growth phases during the 7-day duration of PAM measurements and/or due to the activation of the senescence process. The resulting “zig-zag” shape (dotted lines in Figure 2) should be verified or revised in further experiments. These experiments could take into account, for example, the effects of the absorption of external light inside the leaf vegetation, mutual shielding of leaves, the spectral composition of the light, and the efficiency of electron transport per the PS2 reaction centra.

Hormone analysis indicated a significant effect of plasma treatment on plant hormonal balance. Cell division is generally stimulated by cytokinins. Changes in cytokinin levels suggest that plasma treatment at an optimal duration may have a positive effect on the stimulation of cell division in maize grains (Table 2). Enhanced levels of stress hormones (abscisic acid, salicylic acid, and jasmonic acid) may contribute to the plant’s tolerance to potential stresses.

The presented results suggest that the treatment of grains with Gliding Arc plasma affects the initial growth of seedlings, the metabolism of young plants, and their physiological activities. These results are consistent with previous studies, e.g., [33,34]. Ling et al. [14] stated that NTP treatment may become a fast, economic, and pollution-free method to improve seed performance, plant growth, and ultimately plant yield. The presented data contribute to the characterization of the NTP influence on the germination and initial growth of maize grains and indicate the potential use of NTP in practice.

5. Conclusions

Gliding Arc plasma treatment significantly promoted seed germination and growth parameters of maize seedlings. The highest increase in seed germination (by 41%), root length (by 121%), shoot length (by 198%), and root weight (by 22%) in relation to control samples were recorded for maize grains treated by NTP for 600 s. The NTP treatment of maize grains did not significantly affect the photosynthetic parameters in young leaves. Hormone analysis indicated a significant effect of NTP treatment on plant hormonal balance. While short-term plasma treatment (180 s) had a negative effect on stress hormone levels (abscisic acid, salicylic acid, jasmonic acid, and jasmonate isoleucine) as well as active cytokinins, longer exposure (300 s, 600 s) led to their increase. Above all, cytokinins elevation after longer exposure correlated well with enhanced germination and seedling growth. Auxin levels showed the opposite trend. The achieved data showed that Gliding Arc plasma treatment significantly affected maize grains and may be an effective tool in conventional cultivation systems.

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