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Comparative study of germination in tomato seeds: Plasma-activated water vs. conventional water

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The food sector must pay attention to environmental pollution, resistance to pathogens and climate change, so as to provide healthy feeds for animal and man. Using atmospheric pressure plasma jets (APPJs) is one possible solution. Plasma can enhance germination process, increase agricultural yield, and decontaminate surfaces of food products and seeds. This study is to determine the effects of the plasma-activated water (PAW) produced by plasma jet discharge (PJD) system on in vitro grown tomato seeds. A plasma jet system is developed with a sine wave of an AC voltage peaking 12 kVA peak to peak and ranging to zero, a power of 60 W, and a frequency of 20 kHz. This procedure involves the treatment of 20 cc of distilled water (DW) through Direct Plasma activation, argon gas is supplied at a flow rate of 02 litres per minute. This creates a different pH and levels of reactive oxygen and nitrogen species RONS. The exposure of DW to PJD is done for a duration of five, ten and fifteen minutes to produce PAW. Plasma-activated water (PAW) promotes agricultural sustainability due to its rich content of reactive oxygen and nitrogen species (RONS), making it environment-friendly. This investigation aimed to assess the impact of PAW produced by an atmospheric pressure plasma jet discharge (PJD) system on the germination and early growth performance of tomato (Solanum lycopersicum) seeds. The plasma jet, powered by argon gas at 2 L/min, activated distilled water (DW) while running in a 0–12 kV peak-to-peak, 20 kHz, and 60 W range. PAW is produced by exposing DW to plasma for 5, 10, and 15 min, which affected their concentration of RONS and pH values. The 160 seeds are divided into four groups that consist of three treated groups and one control group. Seven days are observed for germination percentage, germination rate and seedling growth parameters. PAW is significantly positive in germination kinetics and seedling development compared to DW, which is statistically significant ($P < 0.01$). The aforementioned study results indicate that PAW is a sustainable plasma process that improves germination and early growth in tomato seeds.

Keywords: Plasma jet; Germination; Plasma-activated water; Tomato seeds.

1. INTRODUCTION

There is an increased interest in Cold Atmospheric Pressure Plasma (CAPP) due to its potential in sustainable agricultural technologies. The interaction between plasmas and liquids creates water which is reactive towards different chemical species. These species control the process of water uptake, dormancy release and also intracellular signaling during germination. When viewed from a nanotechnology perspective, the PAW is a nano-engineered liquid that reacts and is used to control the interaction with biological surfaces. Even though various benefits have been reported, research on the effects of RONS composition on tomato seeds remains limited. The purpose of this study is the evaluation of PAW effects on tomato seed germination and early growth.

Having various advantageous biochemical and physiological properties [11], enhanced germination [6,9], surface decontamination [10] and faster growth [6,8] are the benefits of preharvest plasma treatment. Several seeds treated with the same plasma that is used in the work presented showed such effect [12,13]. The production of different gaseous and aqueous RONS is a feature of plasma discharge. RONS regulate many activities in various plants and seeds. It includes growth, germination, development, stress response, and more. The main species believed to enhance seed germination and growth of crops irrigated with PAW are RONS; nitrites (NO_2^-), hydrogen peroxide (H_2O_2), and nitrates (NO_3^-) are responsible. Plasma may remove the pathogens from the surface of seeds through mild oxidative stress. If the RONS concentration inside the cell's changes, the signaling pathways that are related to seed germination may change.

A plasma treatment's efficacy is affected by the type of seed, working gas, surface morphology, and plasma source [14]. Due to such factors, it is practically not possible to set uniform plasma treatment parameters for several seeds. Multiple studies [15,16] show that the ideal time of plasma treatment is species-dependent and may be dependent of seed type, size, location of embryo and hardness of seed surface. But there are other problems with plasma treatment as well. Sometimes, there is a prolonged exposure time needed to ensure the inactivation of pathogens, which can damage seeds and reduce germination rates. Seed morphology is severely affected due to the prolonged exposures to plasma which reduce the germination [17].

The increasing relevance of tomato in the world market has led many countries to increase their acreage and export share [18]. Originating from South and Central America, tomatoes are now eaten more in the USA and Europe than anywhere else in the world [19]. Did you know that the global tomato crop area has grown by 164% over the last four decades as comparison consumption which climbed by 314%? Consumption of tomatoes has been increasing at a rate of 3% in recent times. Quality of the products has improved along with the rise in demand.

In this work, a total 60 W plasma jet system characterized by a 20 kHz frequency, a 0-12 kVA peak-to-peak AC voltage sine wave will be used to provide the treatment conditions of seeds of tomato. The method involves directly activating 20 cc of DW with plasma that has variable concentrations of RONS as well as pH. These expediting employs argon gas at a flow rate of 2 liters per minute for treating it which does not produce any adverse effect and the useful effects are retained (such improved germination). Measuring the concentration of RONS in the PAW for improved tomato germination is critical to find practical applications of this technology in agriculture due to RONS being one of the major active plasma components.

1.2 Seeds and germination

The ripe tomatoes are dried, after which the seeds are removed and washed for use. Both in vivo and in vitro seed germination test conducted for PAW impact on seedling growth. To find the germination percentage, we divided the number of seeds that germinated by the total number of seeds in each Petri dish. As the coleoptile sheath and the radicle part emerge from the seed, germination start to take place. When at least a millimeter of the coleoptile the sheath that covers a young shoot of grass or cereal and the radicle the part of the plant embryo that grows into the primary root had emerged from the seed, it is considered germinated.

2. EXPERIMENTAL

The system for treating water is shown in Figure 1. The unique design of plasma is created for the water energization process. The electric power it aims to supply can produce a sine wave with a satisfactory frequency of 20 kHz with 12 kV AC output. In the Teflon Tube which has an internal diameter of 5 mm, a coiled aluminum electrode is put, at a distance of 1 cm from the end. The flow rate of this electrode is 2 L/min, and it is connected to the argon gas regulator and a high voltage. The flow is laminar at this gas flow rate.

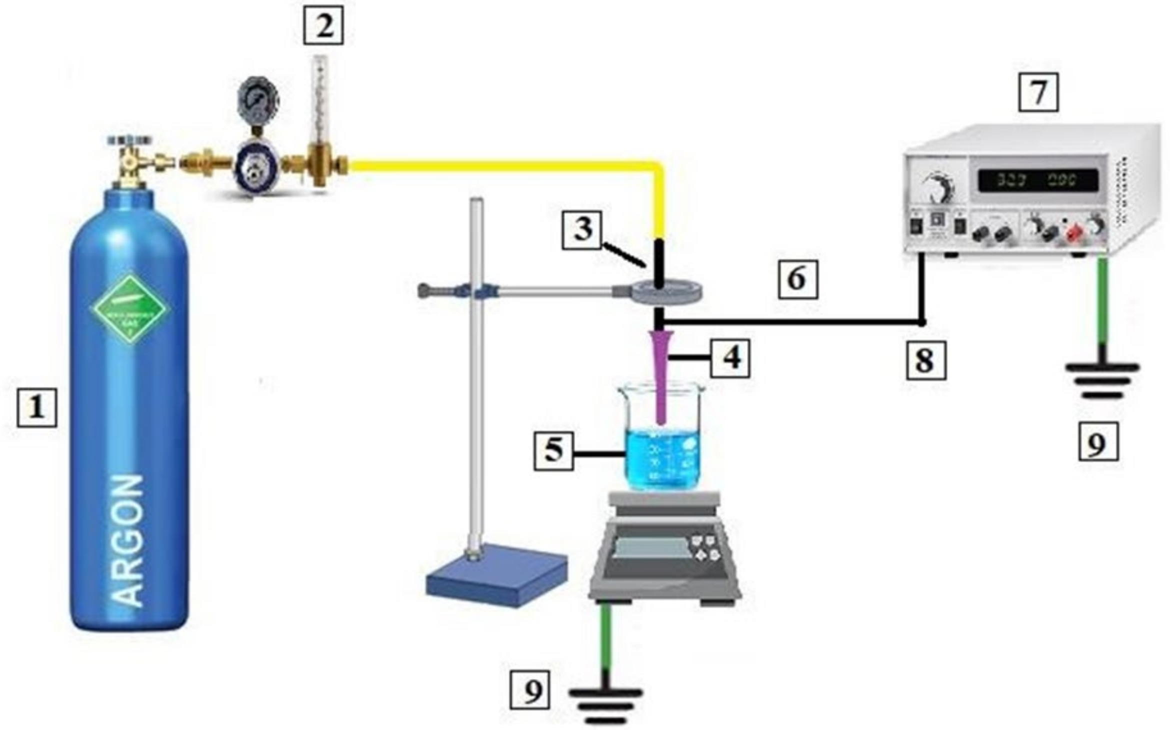

Figure 1 illustrates the Plasma System that has been developed to produce PAW, which consists of 1. Argon gas, 2. Flow meter, 3. Teflon tubing, 4. Plasma jet, 5. PAW, 6. Electrode made of aluminum tape, 7. AC power supply, 8. High-voltage electrode, and 9. Ground electrode.

Figure 2a depicts the plasma system in operation. The water in a 20 mL container is activated by the plasma. The solid plastic colorless tube is a cylindrical vial with a depth of 3 cm and a diameter of 3.5 cm. A plasma is created using a Teflon tube having a headspace of nearly 1.5 cm. To activate DW, plasma is administered for 5, 10, and 15 min at a flow rate of 2 L/min. The PAW in Figure (2b) shows tomato seeds within the plasma system working. The Teflon tube that produced plasma is set above the surface water by 2 cm from the surface of the water to obtain various concentrations of NO3, NO2, and H_2O_2 (i.e., RONS).

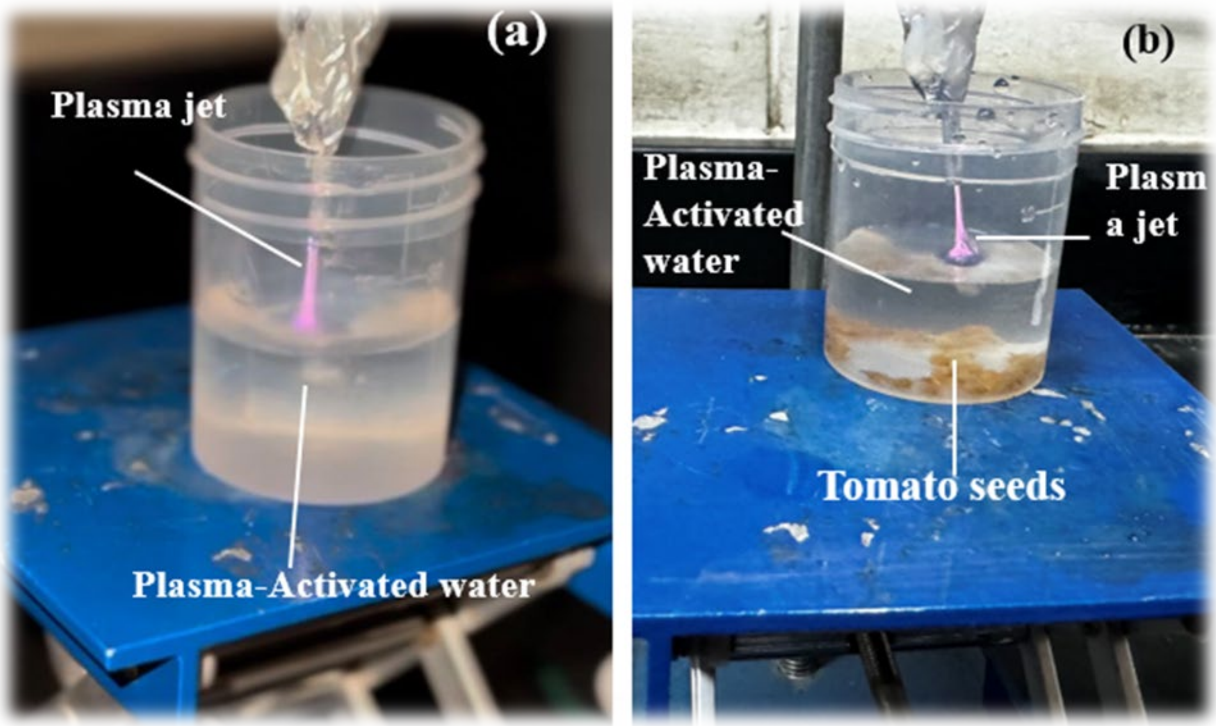

Figure 2 water or tomato seeds activated by plasma system a gas flow rate 2 L/min b.

Test strips (Bartivation, USA) are used to measure the amounts of NO_3, NO_2, H_2O_2, and pH. After immersing the strip in activated water for a few seconds, the excess water is removed to perform the test. As seen in Figure (3), the color of the strip is put to comparison with the standard color following 30 seconds. A remote infrared thermometer is used for recording temperature, and a pH meter is used for measuring pH.

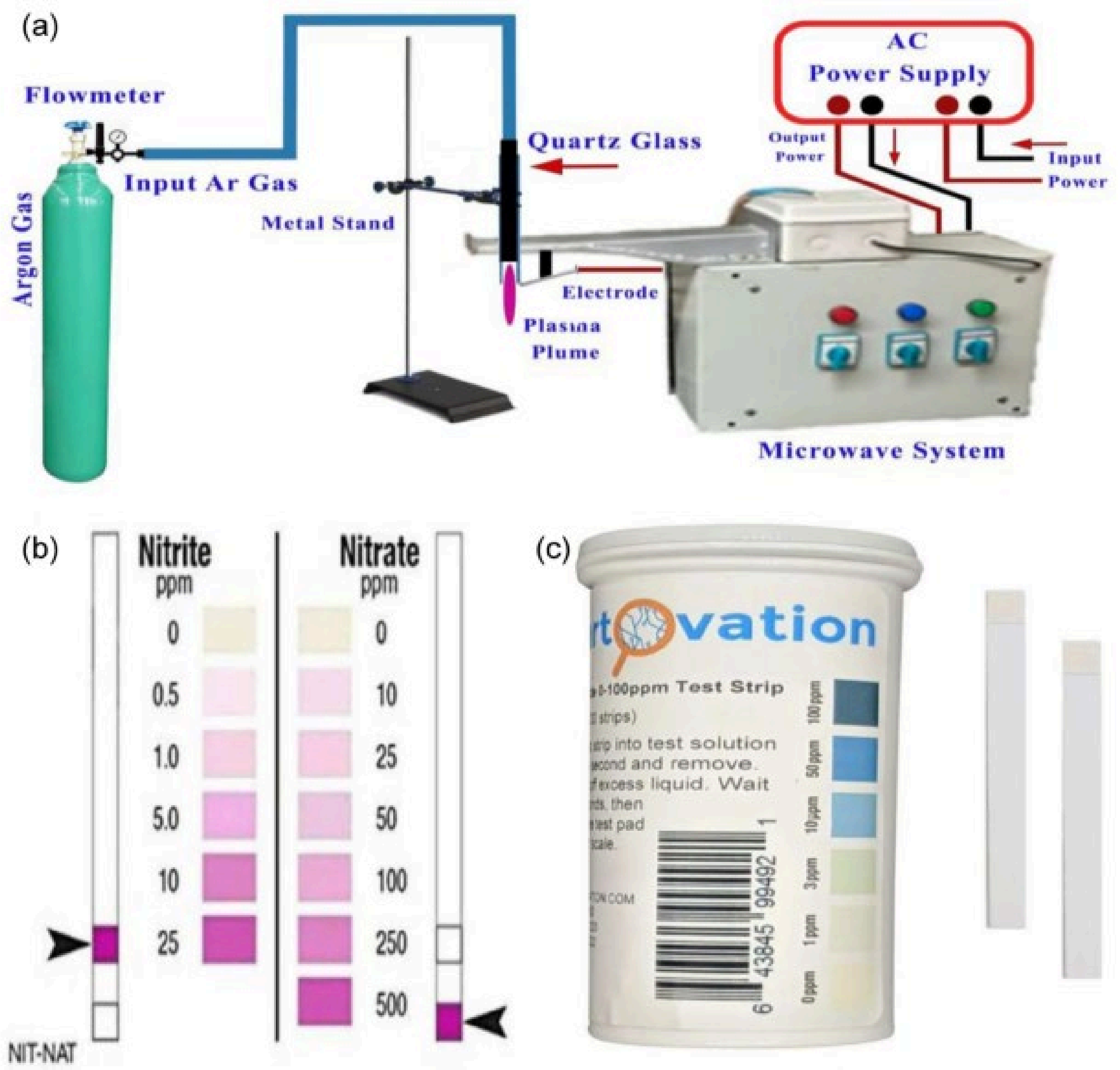

Figure 3 **(a)** Schematic Diagram of the Atmospheric MIPJ System. **(b)** Test Strips Used to Measure NO_2 and NO_3. **(c)** Hydrogen Peroxide (H_2O_2) Test Strips.

A total of 160 tomato seeds are selected for this study and grouped into four batches of 40 seeds. The selected seeds for this purpose are uniform in size, healthy and capable of germination. After washing the seeds well, they are soaked into a chlorine which is inclined with DW for a 10 minutes period. After that, the seeds are washed and dried.

The seeds are randomly assigned into three groups (S1, S2, and S3) which are allotted to DW subjected to plasma with different treatments of various concentrations of NO3, NO2, and H_2O_2 (RONS) (Table 1). The 20 mL active water is used to acquire the concentrations of RONS in the first (S1), second (S2) and third (S3) groups. The S1, S2 and S3 displayed RONS concentration levels approximately 160, 210 and 375 ppm respectively. The purpose of group S0 is to characterize DW that is not turned on by plasma. In Figure 2b, seeds are deposited of DW, plasma treated, and germinated.

In the case of S1, S2 and S3 seeds (Figure 2a), they are soaked for a period of 24 hours in PAW after which they are dried for placement in prepared germination plates. For every group, the seeds are evenly spread on the two plates with 20 seeds each. The keeping dishes are placed over a piece of porous cloth to retain moisture and subsequently covered with a nylon cover for 2 days. Subsequently, on the third day, the cover is removed. The seeds are sprayed daily 3 times for first 3 days of germination. An. environment that had a stable temperature, moderate humidity and continuous artificial lighting is provided to the plates. Over the period of the first to the seventh day of the experiment, the number of seeds that sprouted each day is monitored and recorded. Furthermore, all the commented biological changes in the seeds are also recorded during the period. The researchers conducted similar steps on the S0 group of seeds. However, the seeds of S0 are not exposed to plasma. Over time, the seeds are dried and placed in the germination plates discussed above.

3. RESULTS AND DISCUSSION

The pH and NO_3, NO_2, and H_2O_2 concentrations of the PAW utilized to soak the tomato seeds are displayed in Table 1.

Table 1 Concentrations of NO_3, NO_2 and H_2O_2 (ppm) and pH in the PAW.

Group	NO_2 (ppm)	NO_3 (ppm)	H_2O_2 (ppm)	Total Reactive species (RONS) (NO_2+NO_3+H_2O_2)	pH
S0	0	0	0	0	7
S1	10	100	50	160	6.7
S2	10	100	100	210	5.8
S3	25	250	100	375	5.6

Table 2 Number of germinated and non-germinated seeds over seven days of germination.

Groups	Total number of seeds	Number of germinated seeds on the first day	Number of germinated seeds on the second day	Number of germinated seeds on the third day	Number of germinated seeds on the fourth day	Number of germinated seeds on the fifth day	Number of germinated seeds on the sixth day	Number of germinated seeds on the seventh day	Number of non-germinating seeds at the end of germination
S0	**40**	**1**	**5**	**18**	**34**	**34**	**34**	**34**	**6**
S1	**40**	**5**	**6**	**21**	**35**	**35**	**35**	**35**	**5**
S2	**40**	**3**	**6**	**26**	**36**	**36**	**36**	**37**	**3**
S3	**40**	**2**	**8**	**27**	**37**	**37**	**37**	**38**	**2**

The tomato seeds are soaked in an equal amount of PAW for seven consecutive days during germination, defined as the period in the case when the seedling begins to sprout from seed surface. During the first three days of germination, soaking the seeds in PAW increased the germination speed, as listed in Table 2.

Table 3 Average root and vegetative part lengths of seeds on seventh day of germination.

Groups	Root length (cm)	Vegetative part length (cm)
S0	0.5	1
S1	0.8	1.8
S2	1.2	2.4
S3	1.4	2.5

RONS determined how many seeds germinated. According to the table 3, the average length of seeds as well as vegetative parts on the seventh day of germination for the four groups. Understanding how PAW may alter the germination and development of plants is important from the perspective of the change of chemical due to discharge. Most of the times, NO2 and NO are the main constituents that we observe during the discharge in the ambient air. When they are present with water, the hydroxyl radicals (•OH) are formed and they recombine.to form H_2O_2 (Eq. 1). The discharge of gaseous NO2 and NO in water produces nitrates and nitrites, which makes the water acidic. Table 1 (Eqs. 2, 3) explains how water in which plasma is exposed has a lower pH. In an acidic medium $NO2^-$ could react with H_2O_2 to generate the unstable intermediate (ONOOH) (Eq. 5), which breaks down to produce $NO3^-$ or •NO2 and •OH (Eqs. 6, 7) [20,21] or it could be oxidized to $NO3^-$ (Eq. 4).

$$^{\bullet}OH + {}^{\bullet}OH \rightarrow H_2O_2 \tag{1}$$
$$NO_{2(aq)} + NO_{2(aq)} + H_2O \rightarrow NO_2^- + NO_3^- + 2H^+ \tag{2}$$
$$NO_{(aq)} + NO_{2(aq)} + H_2O \rightarrow 2NO_2^- + 2H^+ \tag{3}$$
$$3NO_2^- + 4H^+ + H_2O \rightarrow 2NO + NO_3^- + 3H_2O^+ \tag{4}$$
$$NO_2^- + H_2O_2 + H^+ \rightarrow O = NOOH + H_2O \tag{5}$$
$$O = NOOH \rightarrow {}^{\bullet}NO_2 + {}^{\bullet}OH \tag{6}$$
$$O = NOOH \rightarrow NO_3^- + H^+ \tag{7}$$

Based on Table 3 showing average root length concerning germinated seeds on the first day, it appears tomato seeds soaked in PAW have a better tendency to sprout than those soaked in DW. The seeds that are soaked in PAW showed healthy germination of their roots; the rootlet lengths for S1, S2, S3, and S0 are about 0.8, 1.2, 1.4, and 0.5 cm, respectively. The rise in growth indicators may be caused by reactive RONS being more concentrated in PAW and remaining stable due to their mutual reactions in Acidic Medium (Eqs. 4, 5). Physiological dormancy could be ended by signaling molecularly members of these groups, NO3-, H_2O_2, NO and •OH. They promote activities that unfreeze the dormancy process i.e. production of gibberellins and breakdown of abscisic acid [22]. The application of PAW affects seed water content, imbibition, and internal transformations [23, 24]. Measurements are taken on the length of the root and of the vegetative portion firstly to measure the most striking change in seeds due to PAW. Referring to the data contained in Table 3, it shows that after the germination period, the average length of the root and vegetative part of the seed of treatment water is significantly longer than in DW (S0). Despite recommendations that the reactive species may be a non-harmful fertilizer for seeds, the authors state that seed germination is linked to the ratios of the reactive species (i.e. RONS). The RONS produced in PAW may have a negative impact on signaling associated with seed dormancy. The result may include stimulation of the germination process and improved germination profile. Most authors claim that the nitrate compounds are taken up mainly by the root system whether a PAW solution is used or the plants are directly watered. Plant growth hormones are the hormones that help in enhancing the growth of plants. The seed strength is the property of the seed that affects seed activity, performance during germination and the development of the root and vegetative parts.

The seeds of tomato in groups S1, S2 and S3 which are directly treated with plasma showed a significantly better germination rate than group S0 (which is soaked in DW) as shown in Figure 4. This enhancement is due to the interaction between the tomato seed surface and RONS in PAW. This has potentially able to increase the germination rates and also the uptake of water and nutrients. The presence of aquaporins and lipid composition of membrane are important for unhindered free diffusion of H_2O_2 from PAW for which the membrane protein which assist water transport is essential200. Although, the transport of NO3- is a slow process. It is facilitated by transport proteins in the membrane rather than simple diffusion and is concentration-dependent. The findings demonstrated that the rate of decomposition of H_2O_2 is faster as compared to that of NO3 and NO2. During imbibition and germination, the seeds enhanced their ability to degrade H_2O_2. Due to cellular signaling and enzymatic activation, the metabolic response of seeds to H_2O_2 is behind the effects of H_2O_2.PAW on seed germination. $NO3^-$ constitutes an important nitrogen and nutrient source for seedlings.

Figure 4 Roots and vegetative parts of tomato seedlings at the end of germination period (8 days).

The germination rate (*GR*), germination ratio (*GP*), and average germination time (*MGT*) have been calculated with the use of equations (8) and (9), with the results presented in Table (4):

$$GP(\%) = \frac{NG}{NT} \times 100 \quad (8)$$
$$GP(\%) = \frac{NG}{NT} \times 100 \quad (9)$$

Where NT is the total number of seeds and NG is the number of seeds that germinated [26]. Di is the number of days, n is the total number of days counted, and Si is the number of seeds that germinated per count [27].

Table 4 Effect of Total NO_2, NO_3 and H_2O_2 Concentrations (ppm) and pH on Germination Indicators: GP and GR.

Group	Reactive species (RONS) (ppm)	Germination percentage (GP)	Germination rate (GR)
S0	Total RONS=0, NO_2=0, NO_3=0, H_20_2=0, pH=7.0	85%	24
S1	Total RONS=160, NO_2=10, NO_3=100, H_20_2=50, pH=6.7	87.5%	30
S2	Total RONS=210, NO_2=10, NO_3=100, H_20_2=100, pH=5.8	92.5%	31
S3	Total RONS=375, NO_2=25, NO_3=250 H_20_2=100, pH=5.6	95%	32

Petri dishes are used to cultivate seeds in vitro. Seed shows comparatively high natural germination rate of 85% to 95% in the seeds. The results of the water treatments with plasma on water melon seeds for seed germination rate and germination percentage is exhibited in Figure 4a as well as 4b. The seed germination of tomato plant is affected by plasma treatment, it shows current result.

The groups with the same letter (a) have a significantly higher value than group S0 with respect to germination speed [24]. According to Figure (5a), maximum stimulating effect and increased 87.5% – 95% germination rate. For the tomato seeds soaked with PAW. Seeds soaked only in distilled water (DW) could germinate up to 85%.

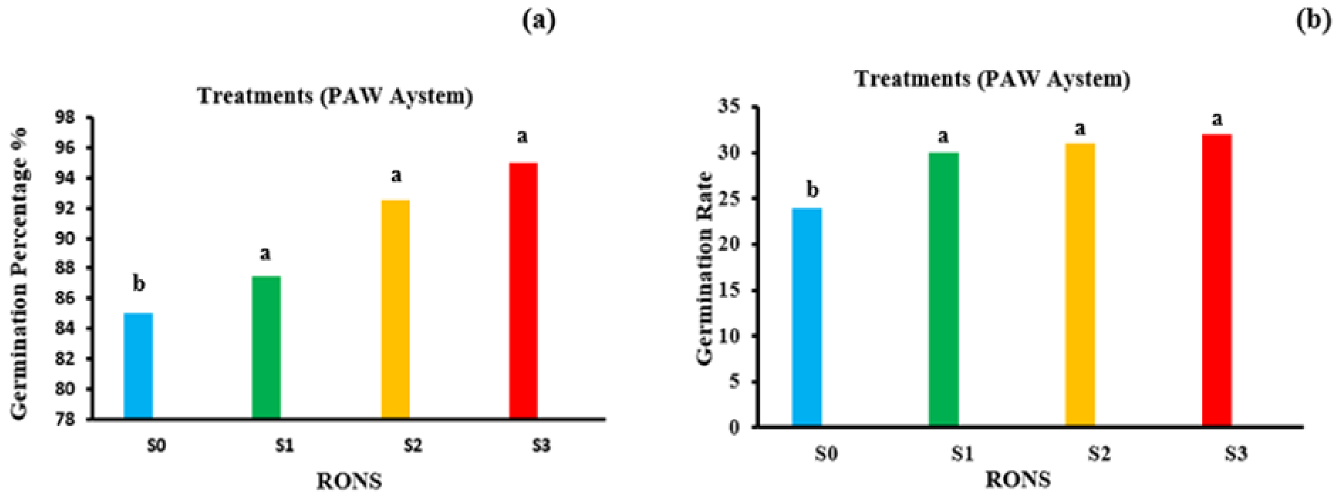

Figure 5 (a) Effect of PAW on Tomato Seeds on Germination Percentage ($P<0.01$) Duncan's multiple range test states that columns with the same letters do not differ significantly Impact of PAW on Germination Rate of Tomato Seeds is significant. According to Duncan's Multiple Range Test, columns with the same letter are not significantly different.

Table 2 display results after analyzing germination of the seed after seven days in vitro culture. Observing the Figure 5b against data given in table 2, it is more noticeable that after 24 hours rise in germination has been more evident than after seven days. Most of the seeds started to germinate during the first 3 days and the variation became somewhat lesser after the fourth day. RONS come from plasma discharges. They alter the oxidation-reduction potential, electrical conductivity and acidity. As mentioned before, this new solution tenders to seed germination rate. Better-hydrated seeds tend to germinate quicker. The average lengths of roots and shoots of seeds after germination are shown in Fig. 6a and 6b. As compared to the control, seeds soaked in PAW gave a higher germination rate and longer seedlings. The interaction of hydrogen peroxide and nitrate caused the endogenous generation of nitric oxide radicals and seed dormancy. The enhancement of uptake of nitrate and other nitrogen ions, and H_2O_2; changes in water pH etc., on which various biochemical changes are going to occur, is said to affect the improvement of germination. Enhancing plasma treatment-promoted assets strongly correlates with improved germination of crops and grounds.

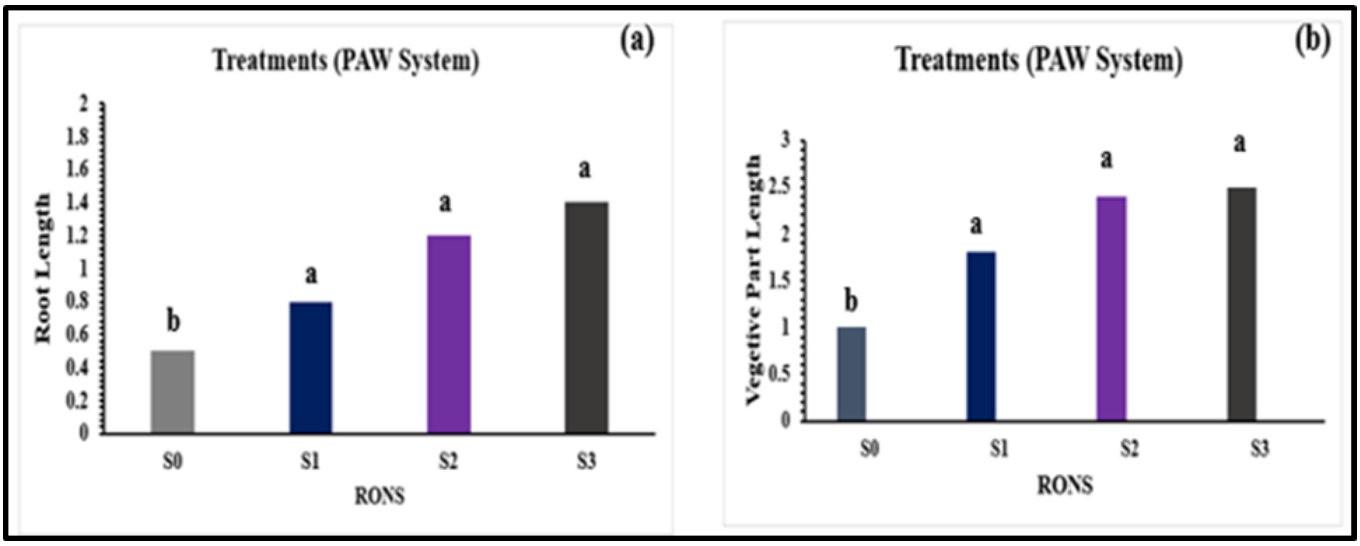

Figure 6a shows the effect of the PAW treatments on the foot length of the tomato seeds. Duncan's multiple range test shows that columns with a common letter do not differ significantly. The plasma-treated water had a significant effect on the length of the growing part of tomato seeds. Columns having the same letter do not differ significantly with reference to Duncan's Multiple Range Test.

The seed germination rate, germination percentage, root length, and shoot length in groups S2, S1, and S3 did not change substantially despite the minor variations in RONS concentrations (Figures 5 and 6). In contrast to group S0, the PAW-treated seeds showed statistically significant improvements.

4. CONCLUSIONS

The study confirms that using plasma-activated water produces significantly higher germination and seedling growth of tomato seeds than distilled water. A water improvement effect is resulting from the controlled physicochemical modification of water through the generation of long-lived RONS and pH reduction through plasma. Middle RONS concentrations gave the best germination responses. In short, PAW is a green plasma-based nanotechnology for agriculture. The next conclusions were drawn. RONS that are stable over time, such as nitrites, H_2O_2 and nitrates, are present in the PAW produced through PJD.

- Soaking seeds in PAW increases the seed germination and early seedling growth.
- The concentration of reactive oxygen and nitrogen species (RONS) as well as their relative ratios may play a role in the enhancement of plant growth, notably the H_2O_2-nitrate ratio.
- The comparison made between seeds soaked in PAW (S2, S1 and S3) and untreated seeds soaked in DW (S0) showed significant improvement. They gave the best results considering growing section rate length, germination percentage, pH decrease, and average root length.
- As opposed to group S0, PAW enhances the growth of tomato seedlings, germination rate, vigour index and water absorption rate.
- Tomato seeds that are exposed to plasma show relatively best results. The application of water-cooling to a plasma generation method using plasma jets that are discharged into water in air bubbles has been successfully implemented to produce any kind of plasma rich in ions and radicals.

According to the research findings, PAW has a high potential for implementation in agricultural processes, from seedlings to harvest stages of plants.

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