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Effect of Plasma-Activated Water with Varying RONS Concentrations on Barley Seed Germination Parameters

The objective of this study will be to reveal the correlation between the concentration of reactive oxygen nitrogen species (RONS) and its impact of seed germination and initial development of a plant. The activation was done by using plasma jet system, with AC voltage sine wave from 0 -12 kVA, frequency 20 kHz and total power of 60 W. The argon gas at flow rate of 2.5 L/m. 60 cm of distilled water was treated with plasma directly. Each 20 cc was treated at different times. 240 seeds of barley were taken and divided equally into four groups. Three groups were treated with water active by plasma WAP and one group was treated with distilled water. After 24 hours the seeds were planted in germination plates. The plates were put in dry room with a normal temperature. The seeds were monitored for four days after soaking process the monitoring focused on germination percentage GP and the germination rate GR. The results were as follows: S1, soaked with PAW, the total RONS concentration of 201ppm GP 93% and GR 53, S2 soaked with PAW the total RONS concentration of 132ppm GP 88% and GR 48.6, S3, soaked with PAW the total RONS concentration of RONS 77 ppm GP 91% and GR 50.6 . The control group GP 85%, and GR 43.3. The statistical calculation indicated that PAW treatment significantly influenced barley seed germination. These findings conclude that WAP positively affects seed germination rates and initial seedling development.

***Keywords*:** Electric discharge; Plasma-activated water; Plasma jet; Reactive species
Received: 29 December 2024; **Revised:** 16 February; **Accepted:** 23 February 2025

1. Introduction

Barley is a versatile food crop and is characterized by a short growing period. It is useful to find ways to improve its productivity, such as chemical and animal fertilizers and soil improvement. These methods are expensive, and the current global trend is to reduce the cost. Therefore, many researchers have conducted many experiments using plasma to eliminate bacteria and enhance production as an alternative to old methods [1]. Similarly, there are some studies which have applied plasma on the soil used for germination to treat seedlings infected with fungal diseases and diseases in the soil [2]. Plasma can be described as the ionized form of the material, which involves atoms, molecules, excited ions, electrons, and free radicals, there are various techniques to generate plasma the most one is electrical discharge in gases. Like Plasma jet discharges, glow discharges, and dielectric barrier discharges are among the common method [3]. The mechanism of using plasma activated water to improve seed germination comes from the following. The interaction of plasma with water and air at the surface of the water leads to the formation of active compounds of oxygen and nitrogen. These compounds have a long half-life and their concentrations can be measured by simple methods, such as NO_2, NO_3, and H_2O_2. There are compounds with a relatively short half-life that require more complex methods to measure their concentration. The presence of these compounds dissolved in water leads to a change in the chemical and physical properties of the water and makes it a killing medium for most pathogens. In addition, nitrogen compounds are a fertilizer in themselves and their presence in water increases the acidity of the water [4]. Cold plasma in the agricultural field can be used in various ways for instance, by either treating or soaking the seed to germinate on PAW. Farah and Hammad investigated how various type of plasma alters the chemical and physical parameters of plasma activated water. Moreover, they investigated on how water storage impacts on NO_2, NO_3, H_2O_2, and pH that are produced in the water [5-7]. Hammad and Tamara where studied the physical and chemical properties of the WAP by plasma jet. The aim of this study was to establish the effects of plasma on the chemical and physical properties of PAW. This was accomplished by analyzing the impact of two factors: the exposure time of the plasma and the flow rate of the argon plasma carrier gas [8]. The technique of using plasma to produce water that is capable of watering agricultural crops is employed when the plasma is used to activate the water that irrigates the crops and or the soil and nutrient media in which seeds are to be planted to sprout and yield better production [9].

However, to improve the germination of soybean seeds, plasma-activated water was used. Soybean seedlings and saplings were subjected to plasma activated water and the effects were studied by Rajesh et al. using dielectric barrier discharge (DBD) [10]. Further, Rajesh et al investigated an application of gliding arc discharge (GAD) to activate water for using it for watering the seedlings. During their experiments

involving seedlings, they found that the rate of germination was higher, and the proportion of the lengths of the shoots and the rate of seed imbibition was also higher [11]. Feizollahi et al. was explorative research that aimed at determining the extent to which plasma activated water can reduce the DON level in barley seeds treated in water [12]. A comparative electro-physiological study on the impact of GD to the germination and growth of plants such as corn and barleys was conducted by Yemeli et al. [13]. In a study by Maxime et al., water was activated using air dielectric barrier discharge plasma and helium plasma jet in a bid to investigate the impact on germination of Arabidopsis thaliana seeds [14]. Some of these seedlings were watered using activated water while others were and their growth and development as they grew plus matured was also monitored and observed [15]. Kučerová et al. discovered that watering wheat seeds with plasma effect on seed by water treated plasma where the plasma created from spark discharge. Include photosynthetic dyes and proteins which are highly soluble than those in the control sample [16].

The considerable part of the previous investigations, which addressed the issue of PAW and germination or germination and growth of seeds, addressed the type of applied plasma and paid insufficient attention to the concentration of RONS as a product of plasma interaction with water. Some of the few studies done on the indication of RONS concentrations employed expensive chemical techniques to quantify the concentration of these species. It takes a long time during examination and is non-conservation friendly, whereas an efficient, direct, and conservation friendly way was adopted in the current study. The current study therefore seeks to establish the correlation on the amount of RONS and its effects on germination of barley, *hordeum vulgare ervan* Iraq seeds and the early growth stage of the germinated seeds.

2. Experimental work

The process of energizing water has been developed with the use of a plasma system. The designed system is presented in Fig. (1). Power supply is an assembly which forms part of the system. It generates sinewave of 20 kHz frequency and has the capability to send big alternating voltages up to 12 kV in a Teflon tube of an internal diameter of 5mm and at a distance of 1cm from the end of the tube an electrode made of coiled aluminum was connected to higher voltage. It is connected to the Argon gas regulator, which flows at a rate of 2.5 L/m and based on this rate of flow of the gas, it is evident that there is laminar gas flow. On the other end of the Teflon tube is the connection end.

The operational plasma system is shown in Fig. (2) as it is seen that plasma work to activate water which is placed in the 30 mL container. The vial is made of hard plastic in shape of a cylinder measuring diameter of 3.5 cm and a depth of 3 cm. It should be noted that plasma was made in the Teflon tube and there was an empty space of approximately 2 cm before the water surface. The distal water is activated by exposing the water to plasma for 20 minutes with a flow rate of 2.5 L/m.

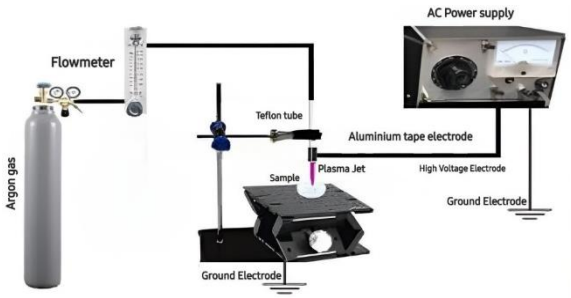


Fig. (1) The developed plasma system for PAW used for seed germination

To obtain different concentrations of RONS, the end of the Teflon tube in which the plasma was generated was placed at a distance of 2 cm from the surface of the water. The concentrations of NO_2, NO_3, H_2O_2 and pH were measured by test strip purchased from Bartvation (American manufacturer). A pH meter was used to measure pH level meter, while the temperature was measured using a remote Infrared thermometer. The test was accomplished by immersing the strip in activated water for several seconds, then excess water remove. After 30 s, the strip color is compared with the stander color as in Fig. (3).

Fig. (2) Plasma system while working to activate the water at Ar gas flow rate of 2.5 L/m

Fig. (3) The strips used in the measurement the concentration of (NO_2, NO_3 and H_2O_2 in ppm)

A chlorine solution was prepared by diluting chlorine in water using distilled water; the prepared solution was used to treat these seeds for ten minutes after washing the seeds. N2000 Barley seeds were used in this study and they were partitioned into four sub groups with each sub group one consisting of 60 seeds. To meet the requirement of the seeds to fit tightly into the holder, those selected was of single size and been healthy as well as germinating capability. Towards the end, the collected seeds were washed and air-dried before progression to the process of cleaning the seeds before disabling. Following a random selection process, these seeds were separated into four groups: The group that will undergo the video intervention will be referred to as Group I (S1, S2, S3), while the group that does not receive the video intervention will be referred to as Control group. Control group was kept in distilled water which did not undergo plasma activation, and water in which S1 was soaked showed 201 ppm of total RONS. In the same way as it was done for the S2, P-AW was used and the total content of RONS in it reached 132 ppm. Another S3 was washed with plasma activated water and this was in total had a measure of 77 ppm of RONS. The seeds are left in the beaker to rest in the plasma activated water for 24 h after which the water is siphoned out and the seeds are allowed to dehydrate and are then transplanted to germination plates that are suited to seed germination. In three plates containing twenty seeds every, the seeds within the plates have been organized within an approach through which all the seeds inside the identical group were positioned together. In three dishes containing twenty seeds each, the seeds in the dishes were placed in a manner that all the seeds from the same group were placed together. It was placed below a section of a clean and transparent clothe through which water could filter for 2 days. The dishes were accompanied by a nylon top. The dressing was changed a little for three days; after that, the covering was removed. This was done in order to track the development of the seeds in terms of the conditions as well as to ensure that there is a sign of growth in the seeds, the seeds should not dry up. Watering is divided into six portions for a plant during the germination process with the span of day one, day two, and day three. This procedure is applied to develop or wet the seeds with a view of germinating them. On the basis of these experiments, it was possible to conclude that the dishes should be preliminarily subjected to a constant temperature, the level of humidity should not be too low or high compared to similar indicators of time of the day, and the meals should be exposed to artificial lighting during daytime and the night.

In the successive observation table, first observation of germination record for the first day, second observation record of the second day and third observation record of the third day was noted down. Moreover, all those changes in the biology aspects of the seeds which occurred during the entire process were photographed and notes were taken.

In this work, 240 seeds of barley were selected, divided into four groups, each group contains 60 seeds, and the seeds were selected to be of one size, healthy and germinable, after washing the seeds well, these seeds were sterilized by soaking them with chlorine diluted with distilled water for 10 minutes. After that the seeds were washed, and dried. These seeds were randomly divided into four groups S1, S2, S3 and control. S1 was soaked with plasma activated water, the total concentration of the RONS was 201 ppm, S2 was soaked with plasma activated water, the total concentration of the RONS was 132 ppm, S3 was soaked with plasma activated water, the total concentration of the RONS was 77 ppm, and the control group was soaked with distilled water not activated with plasma. The seeds were soaked with plasma activated water for 24 hours and then dried and placed in germination plates specially prepared for seed germination. The seeds of each group were distributed on three plates, each plate containing 20 seeds, distributed uniformly. For maintain the moisture of the seeds a piece of porous cloth was placed under these seeds and the dishes were covered with nylon cover for two days, at third day the cover was removed. Seeds are watered in the first three days of germination (6 times a day) by spraying. The dishes were placed where the temperature was stable and the humidity was moderate and normal, and in a place that was lit day and night with artificial lighting. The number of seeds that germinated on the first, second and third day of germination was monitored and recorded, and all the biological changes that occurred to the seeds were recorded and documented with images.

3. Results and Discussion

Table (1) shows the concentration of the RONS (NO_2, NO_3, $H_2O_2$and the pH) in the PAW used for soaking the three groups of barley seeds S1, S2, S3 and the fourth group that was soaked in distilled water.Soaked helped to increase the germination speed as table (2) shows the number of germinating and non-germinating seeds during the three days of germination. From this table, we notice that the highest total germination was for the seeds that were soaked in PAW, and this rate decreased depending on the concentration compared to the control group. Also, the daily seed germination followed the same behavior. Table (3) shows the average length of roots and vegetative of seeds on the third day of germination for the four groups. From the table we notice that, the root of the germinating seeds soaked with activated water showed healthy root. The average length of the roots are for S1=1.5 cm, S2=1 cm, S3=0.7 cm and the Control = 0.5 cm. Table (4) shows the two germination parameters GP the percentage germinations and GR the germination rates. From this table, it can be see that the

highest GP and GR were for the seeds soaked in WAP compared to the control group, and these parameters varied with the concentration of RONS in the WAP. Figure (4) shows the seeds after one day of soaking. Figure (5) shows the vegetative parts and the roots at the end of the third day after soaking as photograph image. From the two figures, we notice a clear effect of activated water on the length and thickness of the roots, as well as on the length of the growing part, compared to the control group. That is because PAW has RONS such as H_2O_2, NO_3^-, $^{\bullet}OH$, and NO which are capable to releasing physiological dormancy by stimulating molecular signaling. It stimulates processes like abscissae acid breakdown and gibberellin synthesis which cause the process of dormancy break [17]. The treatments administered under the PAW regimes affect seed water content, ambition and internal transformation with regard to water absorption influencing germination of barley seeds [17,18]. When (RONS) is postulated to be safe fertilizer for seeds, the seed germination is linked to the ratios of the (RONS). It has been shown that reactive species (RONS) produced in plasma activated water may demonstrate positive signaling interfering with seed dormancy and in this way stimulate the germination process and improve germination profile. Several authors have stated that nitrate compound whether used in PAW solution or in direct watering of the plants are mostly absorbed through the roots and these are known to enhance growth and act as plant growth hormone [19-21].

Table (1) Concentration of the (NO_2, NO_3, H_2O_2 ppm, pH) in the plasma-activated water used to soak the forth groups S1, S2, S3, and the control of the barley seeds

Group	NO_2 (ppm)	NO_3 (ppm)	H_2O_2 (ppm)	Total Reactive species (RONS)	pH
S1	1	100	100	201	5.4
S2	1<	66	66	132	6
S3	1>	25	50	77	5
control	0	0	0	0	7

Table (2) Number of germinating and non-germinating seeds during the three days of germination

Groups	Total No. of seeds	No. of Germinated seeds on the first day	No. of Germinated seeds on the second day	No. of Germinated seeds On the third day	No. of non-germinating seeds At the end of third day
S1	60	49	3	4	4
S2	60	42	4	7	7
S3	60	45	4	6	5
control	60	38	2	11	9

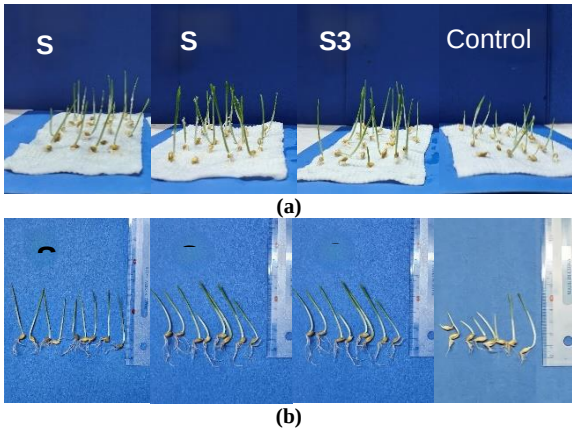
Table (3) Average length of roots and vegetative of seeds on the third day of germination

Group	Root length (cm)	vegetative part length (cm)
S1	2.7	4.5
S2	3	4.2
S3	2.3	4.7
Control	1.7	4

Table (4) The Effect of total NO_2, NO_3, H_2O_2 concentration ppm and pH on the germination parameters GP and GR

Reactive species (RONS) (ppm)	The germination Percentage (GP)	Germination Rate (GR)
S1 Total (RONS) =200, NO_2=1 NO_3=100, H_2O_2=100 ,PH=5.4	93 %	51.8
S2 Total (RONS) =132, NO_2 =>1 NO_3=66, H_2O_2=66, PH=6	88 %	46.3
S3 Total (RONS) =77, NO_2 =<1 NO_3=25, H_2O_2=50 , pH=5	91 %	49
Control Total (RONS) =0, NO_2 =0 NO_3 =0, H_2O_2 =0, pH =7	85 %	42.6

Fig. (4) Image of the seeds of the four groups after one day of soaking



(a)

(b)

Fig. (5) (a) Image of the vegetative part at the end of the germination period after four days (b) Image of the root and vegetative parts at the end of the germination period after four days

Germination percentage (GP) and germination rate (GR) were calculated by equations (1) and (2) [22]

$$GP = \frac{NG}{NT} \times 100\% \quad (1)$$

where NG is the number of germinated seeds; and NT is the total number of seeds

$$GR = \sum_{i=0}^{n} \frac{Si}{Di} \quad (2)$$

where Si is the number of germinated seeds per count, and Di is the number of days and n is the number of days that have been counted [23,24]

Figures (6-9) show the statistical result of the barley seeds socked in WAP. For germination percentage, germination rate and the growing part as well as the root length the present statistical result indicated that socked seeds in WAP affect the GP, and the growing part as well as the root length of barley seeds. There is statistical sufficiency and a significant difference between these indicators and the control group. Except GR has no statistical validity. However, the observed variations in germination parameters across different RONS concentrations also suggest that an optimal concentration range exists for maximizing the beneficial effects of PAW. The PAW has RONS such as H_2O_2, NO_3^-, - OH, and NO which are capable to releasing physiological dormancy by stimulating molecular signaling. It stimulates processes like abscissae acid breakdown and gibberellin synthesis which cause the process of dormancy break. Several authors have stated that nitrate compound whether used in PAW solution or in direct watering of the plants are mostly absorbed through the roots and these are known to enhance growth and act as plant growth hormone [19-21].This highlights the importance of carefully controlling the RONS concentration in PAW treatments to avoid potential phytotoxic effects. Moreover, the observed increase in root length in PAW-treated seeds further supports the notion that PAW can promote early seedling vigor. A healthy root system is crucial for nutrient uptake and overall plant establishment, suggesting that PAW treatment can provide a valuable advantage during the critical early stages of plant development.

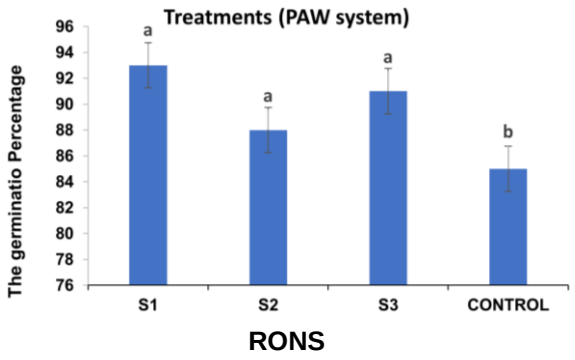


Fig. (6) Effect PAW on germination percentage of barley seeds (P-value = 0.137). Columns with the same letter do not significantly differ according to Duncan's multiple range tests; PAW

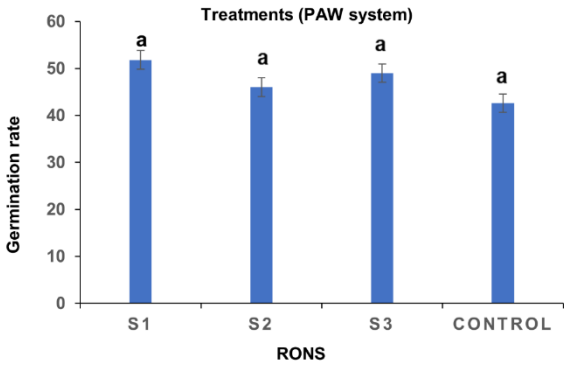


Fig. (7) Effect of PAW on barley seeds germination rate (P-value = 0.003). Columns with the same letter do not significantly differ according to Duncan's multiple range test

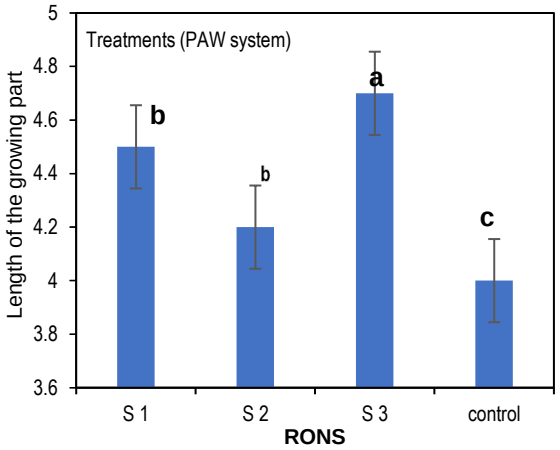


Fig. (8) Effect of water treatments using plasma on the Length of the growing part for the barley seeds (P-value <0.001) Columns with the same letter do not significantly differ according to Duncan's multiple range test

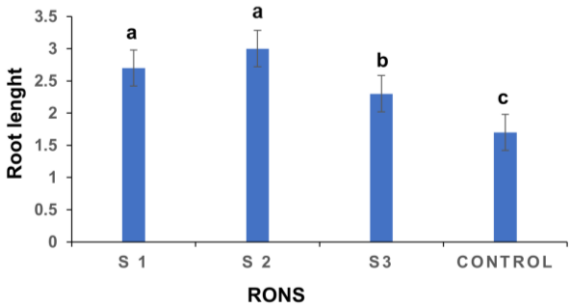


Fig. (9) Effect of water treatments using plasma on the root length of the barley seeds (P-value <0.001) columns with the same letter do not significantly differ according to Duncan's multiple range test

4. Conclusions

Soaking barley seeds in PAW resulted in higher total germination compared to the control group. The effectiveness of PAW in seed germination varied with concentration, of RONS and the highest concentration of RONS was not necessarily the best for seed germination, indicating there is an ideal concentration. Statistical calculations confirmed that PAW treatment had a significant effect on barley seed germination, indicating a positive correlation between PAW

treatment and improved germination results. These results indicate that PAW can be an effective and applicable method for improving barley seed germination. These findings suggest that PAW can be a viable and effective method for enhancing barley seed germination, offering a potential alternative or supplement to traditional seed treatment methods.

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