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ORIGINAL PAPER

Quad-atmospheric Pressure Plasma Jet (q-APPJ) Treatment of Chilli Seeds to Stimulate Germination

Naeem Ahmed[1,2] · Asad Masood[1] · Rubab Mumtaz[3] · M. F. Mohd Razip Wee[1] · Kok Meng Chan[4] · Anuttam Patra[5] · Kim S. Siow[1]

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Abstract

In the current study, a square assembly of four quad-atmospheric pressure plasma jets (q-APPJ) is used to treat large-sized chilli seeds simultaneously. Germination and growth characteristics improve significantly after a 10-sec treatment of q-APPJ employing argon as the working gas. Plasma-treated chilli seed is more etched and porous than those untreated seed surface, as shown in scanning electron microscopy. The chemical changes of the plasma-treated seeds showed that the Ar plasma-treatment oxidise the seed surface to enhance their wettability, stimulate the water uptake, increase the water electrical conductivity and result in improved seed germination. In addition, optical emission spectroscopy is used to detect the different plasma species present and evaluate their plasma parameters (electron temperature and density). These positive results suggested that Ar plasma-treatment, in APPJ setup, improve seed germination, and potentially improve crop yield, and food security issues.

Keywords Active Species · Chilli Seeds · Germination and Growth · Plasma jet · plasma-treatment

✉ Kim S. Siow
kimsiow@ukm.edu.my

1 Institute of Microengineering and Nanoelectronics (IMEN), Universiti Kebangsaan Malaysia, UKM, Bangi 43600, Selangor, Malaysia

2 Molecular Electrochemistry Laboratory, Institute of Fundamental and Frontier Sciences, University of Electronic Science and Technology of China, Chengdu 610054, China

3 Department of Physics, Quaid-e-Azam University, Islamabad, Pakistan

4 Petroliam Nasional Berhad, PETRONAS Twin Towers, KLCC, Kuala Lumpur 50088, Malaysia

5 Chemistry of Interfaces Group, Luleå University of Technology, Luleå 97187, Sweden

Introduction

Plasma, the 4th state of matter, is generated by the ionisation of gases [1]. Plasma is divided into cold and hot plasma, also known as non-thermal and thermal plasma, based on their temperature ranges, respectively. The electron temperature in thermal plasma is like the heavy particles (ionic and neutrals), resulting in extremely high electron densities of the order of 10^{21} m^{-3} to fully ionised levels. This contrasts with the non-thermal plasma, which results in electron temperatures being substantially higher than those of heavy particles, and their electron densities are normally below 10^{19} m^{-3} [2, 3]. Furthermore, this non-thermal plasma is further classified into atmospheric-pressure and low-pressure plasma.

Atmospheric-pressure plasma jet (APPJ) is one of the most common configurations of this atmospheric pressure plasma, and hence widely researched for diverse applications during the last few decades [4–6]. Arc-based APPJ was initially explored for commercialisation as early as the late 1950s [3]. In the late 1980s, the migration from low-pressure plasma processes to atmospheric-pressure conditions gathered momentum to reduce reliance on costly vacuum facilities in the low-pressure plasma [3]. Towards the end of the 1990s, new theories and designs on producing APPJ without requiring a transferred arc materialised with the use of pointed electrodes similar to those used in corona discharges. This new design used dielectric materials to cover at least one electrode to reduce the discharge region while pulsing the discharge and using alternating voltage signals.

Several plasma discharges have been demonstrated to date, with the plasma jet being the most widely used. Plasma gas (also known as ionisation gas) is fed into the region where ionisation occurs by various mechanisms in a typical plasma jet. Afterwards, plasma ions flow through a jet head and extend into the surrounding environment to treat the target materials [1]. Plasma jets can be easily modified to fit the needs of various applications and treatment outcomes because of multiple variables available to control the plasma jets. Some of these variables are electrode types and dielectrics, precursor vapour, working gas, gas flow rate, power type, and quantity of power employed [1]. Different geometries for plasma jets were also available based on the electrodes and dielectric types [3, 7].

At the same time, the development of plasma technology coincides with the development of agriculture technology in improving seed quality and crop yield. Non-thermal APPJ were successfully utilised for the germination and growth improvement of different seeds, resulting in continuous interest in this technology [8, 9]. Simultaneously, cultivable lands reduce with increasing urbanisation and population, and therefore, one possible solution to increase food production is to use this plasma-based agricultural technology to enhance seed germination and yield [10, 11]. Amongst the different crops, chilli is an important food crop cultivated around the world, significantly contributing to global food and economic value. This crop is commonly produced and consumed in South Asia, Southeast Asia, Latin America, the Korean Peninsula, and Malaysia [12]. In addition to serving as a food, condiment, and nutrients in terms of calcium and vitamins, chilli is also used for medicinal purposes [13]. These various beneficial traits of chilli encourage its commercialisation and plantation.

However, the plant development of chilli was limited by poor and irregular germination at its early growth stage [12, 13]. To overcome the dormancy and improve the germination of chilli seeds, various methods have been utilised with different degree of successes. For example, priming strategies to enhance chilli germination include chemical seed digestion and halo priming [14]. In a recent study, low-pressure oxygen plasma was also utilised to enhance chilli seed germination and growth [15]. However, the priming processes may alter the taste of fruits, while low-pressure plasma necessitates the use of vacuum, which is expensive and time-consuming. Therefore, a low-cost and effective approach is required to enhance the germination and growth of the chilli seeds.

In the current study, a square assembly of four (quad) atmospheric pressure plasma jets (q-APPJ) was created by jet-to-jet coupling of four jets to enable plasma to treat a wide surface area in a short time. This q-APPJ operated on the same principles as the simple plasma jet, but its exposure capacity was approximately four times that of the single tube jet. The chilli seeds were treated with this q-APPJ using Ar as the working gas to enhance germination and growth. Water uptake, water electrical conductivity (EC), Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM) studies were used to evaluate the physical and chemical changes of the chilli seeds exposed to Ar plasma-treatment. In addition, optical emission spectroscopy (OES) was utilised to investigate the plasma generated species required for germination and, also to calculate the corresponding plasma parameters (electron temperature and density).

Materials and Methods

The Source of Seeds

Malaysian Agricultural Research and Development Institute (MARDI) provided the chilli seeds (variety Semerah and batch 10/16/JK/B3 14,032,017 from *Capsicum annuum L.*) Healthy seed samples with no sign of disease were selected and used in the current study. Quartz tubes, Teflon solid rods with a diameter of 50 mm, copper electrodes, and power sources were purchased from the local market and fabricated according to dimensions in Fig. 1.

Atmospheric-pressure Plasma Jet

Figure 1 shows the plasma setup used in the current study. The plasma discharge was produced in a plasma jet consisting of four quartz tubes. A copper wire (1 mm diameter) was put into each quartz tube which was connected to a high voltage (HV) and acted as an HV electrode. The outside electrode, fastened around the quartz tube, served as a grounded electrode which was also made from copper. All four quartz tubes were fitted into a Teflon rod of 50 mm diameter. Finally, all HV electrodes were joined together to form a single electrode that was connected to the power supply.

Each quartz tube has its own working gas from soft tube made of polyvinyl chloride (PVC). These PVC tubes were joined with a T-joint to form a single tube attached to a mass flow controller and finally with a gas source. The outer and inner diameters of the quartz tubes were 5.0 mm and 3.0 mm, respectively. The length of the quartz tube and Teflon rod were 15 cm and 8 cm, respectively. The distance between the HV electrode tip and the exit nozzle for the quartz tube was maintained at 10 mm throughout the experiment. The exit nozzle of the quartz tube was 20 mm away from the chilli seed samples. Mass flow controllers were used to feed the Ar (99.9%) pure into the system at a flow rate of 40 sccm. An electronic neon power supply (model: HB-C02TE, Hongba, Hyrite Lighting Co.) with a

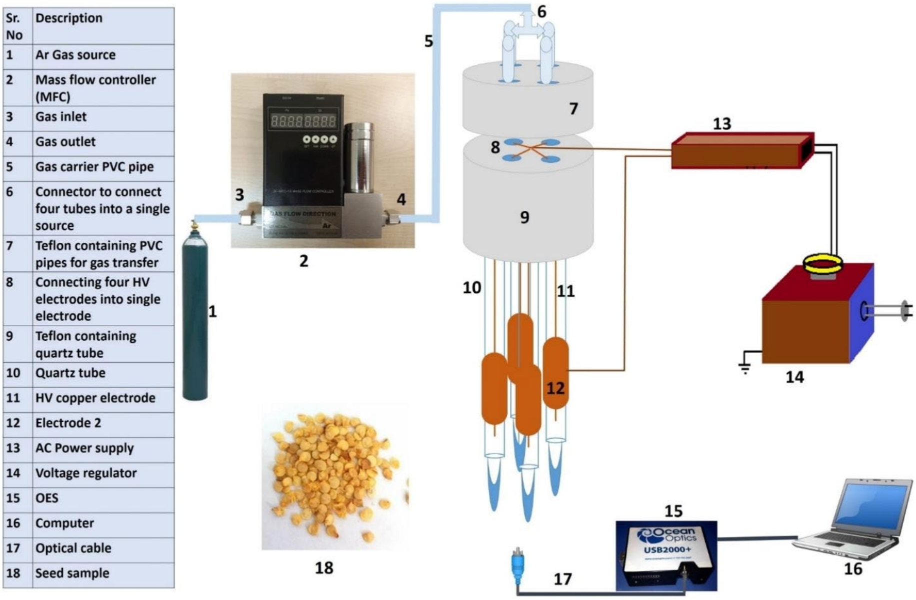


Fig. 1 Schematic of the experimental setup used to treat the chilli seeds for germination and growth improvement and to investigate the plasma parameters (electron temperature and density) of the q-APPJ

maximum voltage of 3 kV and frequency of 40–48 kHz was used to power this Ar plasma jet.

Plasma Treatment

Chilli seeds were exposed to the Ar-based plasma for 10 s. According to International Seed Testing Association (ISTA) rules, a total of 400 chilli seeds were used for one sample in an experiment [16]. Each experiment was carried out thrice.

Germination of Seeds

All germination tests complied with the ISTA-based techniques employed previously [10, 17]. In brief, chilli seeds were grown on tri-layer tissue paper in an airtight plastic container to retain humidity during germination. Each germination experiment employed 800 seedlings (400 for untreated and 400 for plasma-treated), and the experiments were performed in triplicates. Then, 8 square boxes with 100 seeds each were used to germinate the chilli seeds. 10 to 15 mL of de-ionised (DI) water was sprayed into the container to preserve the humidity every 24 h. The lengths of the seed's roots and shoots were also measured on the 21st day. The root and shoot lengths of ten randomly selected plants were measured with a vernier calliper to an accuracy of 0.01 cm on the grown seeds that were stretched straight on a black sheet. Minitab 17 software was used to statistically analyse the germination results; the means were statistically significant when $p < 0.05$.

Water Uptake and Electrical Conductivity

The water uptake and water EC of chilli seeds were calculated based on previously reported protocol [18]. For testing water uptake and EC, 0.2 g of chilli seeds were weighed using an electronic scale to an accuracy of 0.1 mg, submerged in 10 mL of DI water for 24 h, and then reweighed. The increment was then normalised per unit of grams. The EC of the water containing untreated and plasma-treated water was measured with a conductivity meter (model CTR-007, manufacturer Fuzhou Centre).

Scanning Electron Microscopy (SEM)

The surface study of the plasma treated and untreated chilli seeds was investigated with an SEM (model: JEOL JSM-6510LV). The instrument operates at an accelerating voltage of 15.0 kV using the secondary electron mode.

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectra of the seed surfaces were collected using an FTIR spectrophotometer (Perkin Elmer Frontier, model C89399) equipped with an attenuated total reflection (ATR) accessory having diamond crystal. Spectra were obtained in the range of 4000 – 500 cm^{-1} with an average of 16 scans accumulated [19].

Optical Emission Spectroscopy (OES)

The previously described technique was used to conduct the OES study [15]. The plasma species created during the plasma-treatment were identified using Ocean Optics USB2000. In the dark, a 727-733-2447 optical cable was situated 5 cm from the plasma jet to capture the spectra and analysed with Ocean View software (version 2.0.7). The plasma parameters (electron temperature and density) were calculated based on the wavelength and intensities values from OES spectra, while other values (like statistical weight etc.) corresponding to wavelengths were taken from the NIST database.

Results and Discussion

Square Assembly of Four Jets and Plasma Diagnostics Using OES

The development of plasma jets for use in agriculture is an ongoing initiative worldwide and is generally based on the dielectric barrier discharge (DBD) principle. These plasma jets were used to sterilize seedlings as well as to increase seed germination and growth because of their ability to deliver a variety of plasma-derived species under different operational parameters to effect these changes [4]. Here, a q-APPJ (as shown in Fig. 1) was designed to treat a larger sample area than a single plasma jet.

This plasma geometry was successfully demonstrated to treat the agricultural chilli seeds to improve germination and growth (discussed in the following sections). In addition, this plasma geometry can be easily used in other plasma applications without the restriction of vacuum required in a low-pressure plasma facility. The plasma diagnostic was carried out using an OES to identify the active species generated during plasma discharge and determine the plasma parameters (electron temperature and density). Figure 2 depicts the OES measured between 250 and 950 nm. The dominant Ar and oxygen species most likely etched the seed coats; nitrogen species were likely to arise during air discharge at the plasma jet tip. These results corresponded with similar studies from active ion species etching artichoke seeds, that result in enhanced germination [20]. These surface chemical modifications, to be discussed in the FTIR section, on the surfaces of our chilli seeds facilitated water absorption, which aided the seed germination.

Using the remote diagnostic method, OES is used to calculate the electron density and electron temperature of Ar plasma [21]. OES collected and focused this visible light on the slit of the spectrometer without any perturbation in the plasma spectra. This emission spectra from the Ar plasma revealed the temperature and density of the electrons. While there are several ways to use OES to calculate the electron temperature, the method that relies on the relative intensities of two spectral lines belonging to the same atomic species is preferred because this method allows for the calculation of the electron temperature without having to know the number density of the atomic species. Here, Eq. (1), illustrates the formula for calculating electron temperature (T_e) [22, 23].

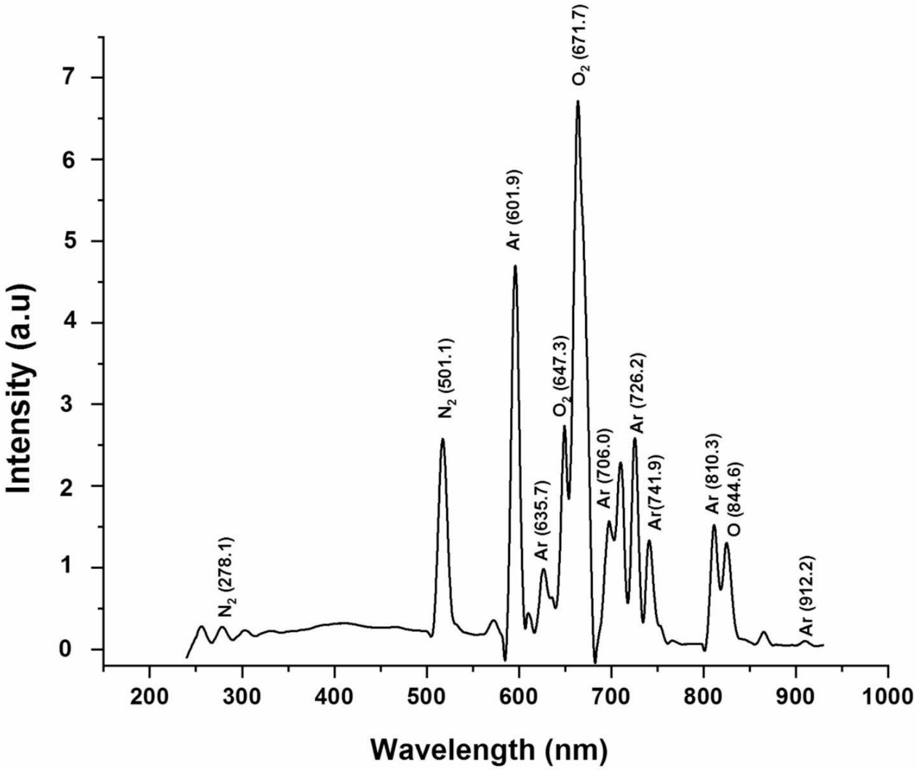


Fig. 2 OES spectra of the q-APPJ show emissions of excited species between 250 and 950 nm

$$T_e = \frac{E_2 - E_1}{k\ln(I_1 A_2 g_2 \lambda_1) - k\ln(I_2 A_1 g_1 \lambda_2)} \quad (1)$$

Where I_1, λ_1, g_1 and A_1 are the total intensity, wavelength, statistical weight, and transition probability, respectively, of one Ar line with E_1 its energy. The corresponding quantities for the 2nd Ar line are I_2, λ_2, g_2, A_2 and E_2. Using the intensities of two Ar-I lines (706.7 and 810.3 nm), the electron temperature of the Ar plasma was calculated. The intensity of these lines was taken from the spectrum recorded using USB2000 OES from the range between 250 and 950 nm. Wavelengths, intensities, transition probabilities, energies, and statistical weights in Table 1 are taken from the NIST (Atomic Spectra Database) and confirmed with the published literature [22–26]. The electron temperature was calculated to be 1.4487 eV using the values from Table 1 and the Boltzmann constant (k) having a value of 1.3807×10^{-23} Joules per Kelvin in equation number (1).

The electron number density (n_e) was calculated using the intensity ratios of two Ar spectral lines (706.7 and 810.3 nm) using the Boltzmann and Saha equations, as shown in Eq. (2) [23, 25].

$$n_e = \left(\frac{2\pi m_e k}{h^3}\right)^{3/2} \left(\frac{2I_2 A_1 g_1 \lambda_2}{I_1 A_2 g_2 \lambda_1}\right) exp\left(\frac{E_2 - E_1}{kT_e}\right) \quad (2)$$

Based on this Eq. 2, the electron density was calculated to be 3.4867×10^{15} cm^{-3} using the values from Table 1 and the Planck's constant (h) having a value of 6.62607015×10^{-34} J-seconds in equation number (2).

Water Uptake Analysis and Seed Germination

Plasma treatment modifies the surface of the plant seeds chemically and etches their surfaces physically to promote nutrient and water diffusion to enhance germination and growth improvement [10]. Figure 3 demonstrates that the Ar-based q-APPJ significantly enhanced the germination potential (%), germination percentage (%), root length, and shoot length of chilli seeds compared to the untreated seeds. The initial count (day 8) and the final count (day 22) were referred to as germination potential and germination percentage, respectively.

Hydrophilicity, surface oxidation, and interaction of active plasma species with seed surface play synergistic roles in improving the seed's germination [27, 28]. In addition, the general seed germination process may also be influenced by the modifications in seed surface wettability, improved water uptake, and modifications in hormonal activity [27, 28]. These mechanisms may be similar to those reported in *Andrographis paniculata* seeds treated with air plasma [29]. In our study, germination was observed for four weeks; however, the germination percentage in the 3rd and 4th weeks was the same. Visually, Fig. 4

Table 1 The wavelength, intensity, energy, transition probity, and statistical weight are used to calculate the plasma parameters

	Line 1	Line 2
Wavelength (nm)	$\lambda_1=810.3$	
Intensity (W/m^2)	$I_1=784.3$	$I_2=683.3$
Energy (eV)	$E_1=1.5$	$E_2=1.7$
Transition probability (s^{-1})	$A_1=2.5$ e^{07}	$A_2=3.8$ e^{06}
Statistical weight	$g_1=3$	$g_2=5$

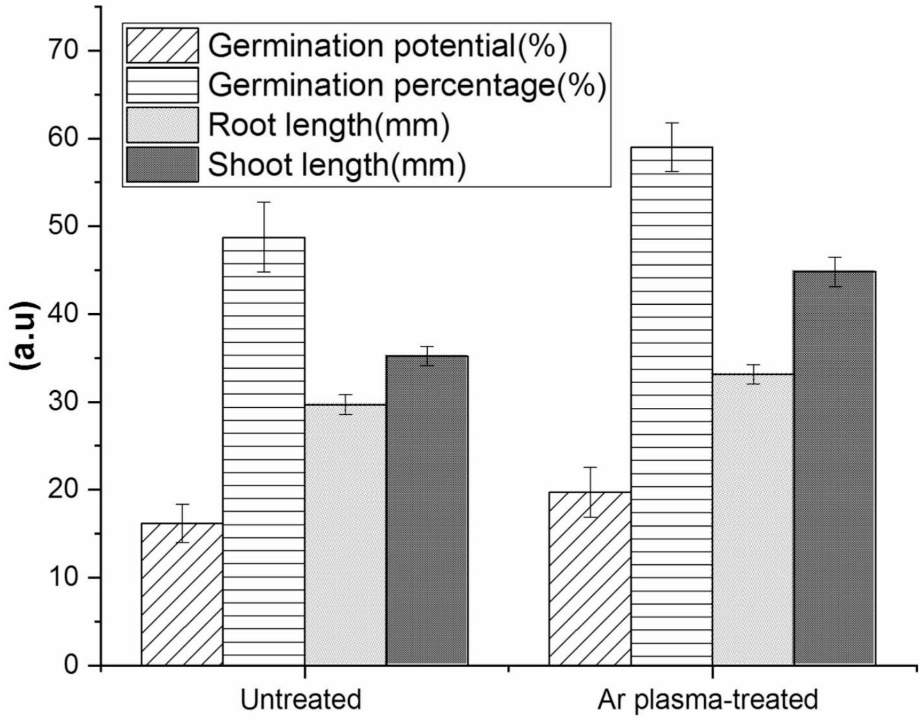


Fig. 3 Germination potential, germination percentage, root length and shoot length of untreated and Ar plasma-treated chilli seeds. The standard deviation of the three experiments performed is represented by the error bar

compares the plasma-treated and untreated chilli seeds grown in the same container with similar environmental conditions. The Ar plasma-treated seeds germinated and grew significantly better than those shown for the untreated seeds.

Water Uptake and Electrical Conductivity (EC)

The ability of seeds to adsorb water is an effective predictor for seed germination. According to Fig. 5(a), the water uptake of chilli seeds is significantly increased after plasma-treatment with the atmospheric-pressure Ar plasma. Similarly, as shown in Fig. 5(b), the EC of solutions containing atmospheric-pressure Ar plasma-treated seeds was much higher than those reported for the untreated seeds.

Water uptake by the seed coat or hilum is the first phase of germination and that uptake initiates the hormone-driven transfer of nutrients essential for growth. Increased water uptake promotes nutrient uptake and provides the energy needed to begin germination. In other instance, seeds exposed to high-intensity plasma or chemical treatment may have damaged cell walls, allowing electrolytes to seep out. In our scenario, the seeds absorbed the activation solution for the growth hormone and nutrients to stimulate germination and plant growth [10]. These current findings indicated that, among other things, greater water consumption and water EC aid the early phases of germination.

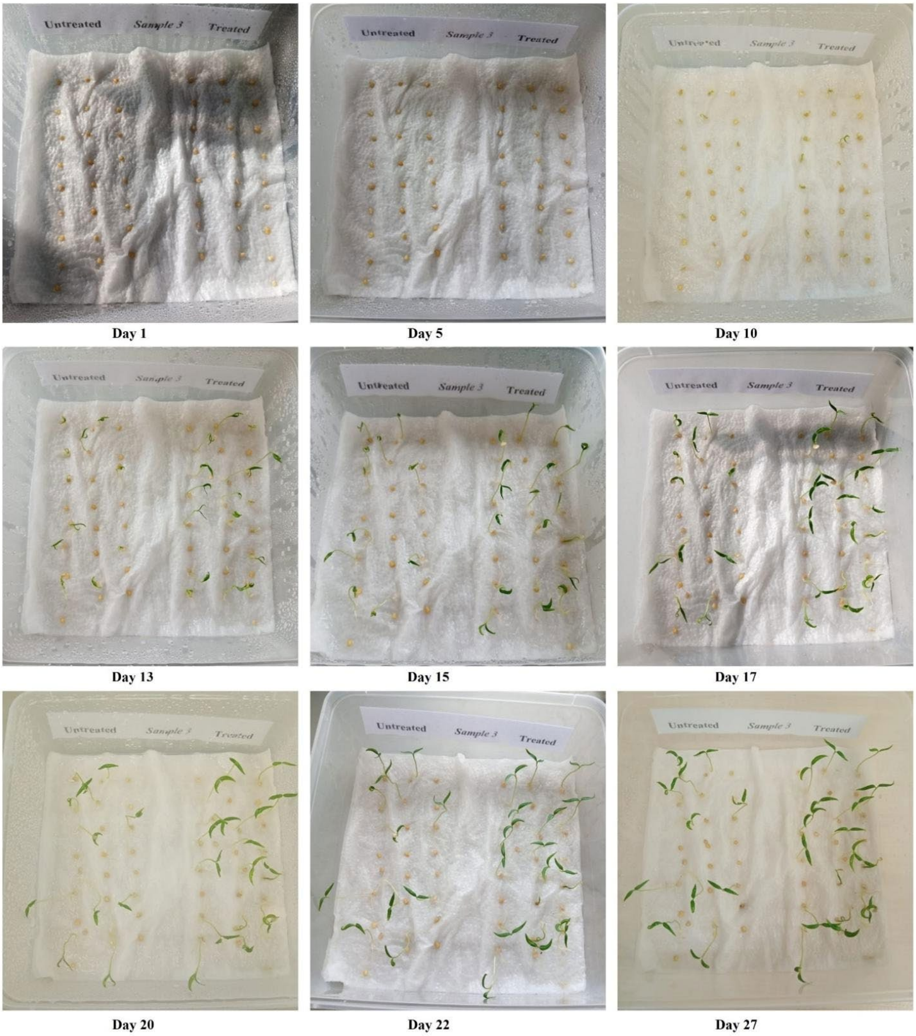


Fig. 4 A comparison of plasma-treated and untreated chilli seeds grown in the same container with the same environmental condition

Surface Morphology

SEM studies showed physical changes after Ar plasma-treatment on chilli seeds. As shown in Fig. 6, the plasma-treated surface is more etched and porous than the untreated surface. The active plasma species that came into interaction with the seed surface resulted in the etching of the seed coat. Our findings are consistent with those reported when the seed coat and hilum of seeds were plasma treated with low-pressure Ar plasma [30]. Tong et al. noticed the same physical changes in *A. paniculata* when it was treated with atmospheric-pressure air plasma [29]. Significant physical and chemical changes in seed surfaces accelerated the imbibition processes compared to untreated chilli seed. This increase in water

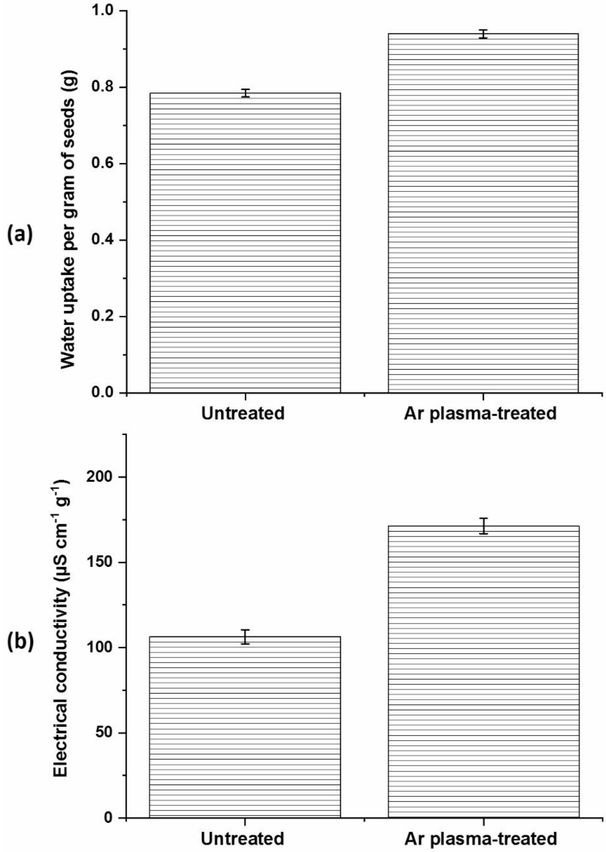


Fig. 5 The (**a**) water uptake and (**b**) water electrical conductivity values of untreated and Ar plasma-treated seeds, respectively. The standard deviation of triplicate experiments performed is represented by the error bar

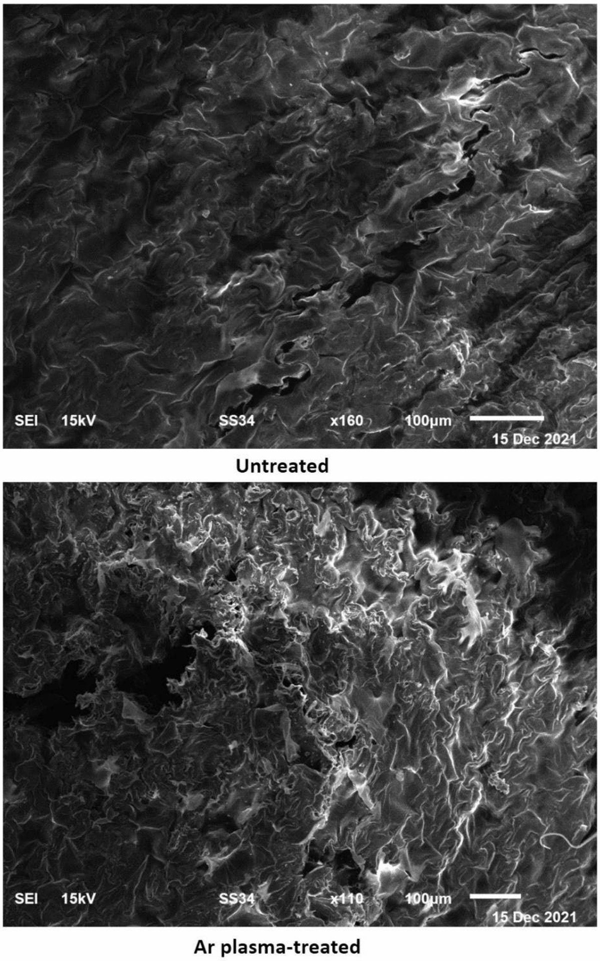
uptake and wettability was consistent with our previously reported results on water uptake and water EC work for other crop seeds [10, 18, 31].

Fourier Transform Infrared (FTIR) Spectroscopy

An FTIR spectrophotometer was used to identify the chemical changes induced on the seed's surface by the Ar-based APPJ. Different bands O–H (3500−3300 cm^{-1}), C–H (2900−2800 cm^{-1}), C=O (1800−1500 cm^{-1}), C–O–H (1500−1200 cm^{-1}) and C–O (1200−900 cm^{-1}) were visible on the chilli seeds after the Ar plasma-treatment, as shown in Fig. 7 [32, 33].

The bands in the 1800−1500 cm^{-1} region are characteristic of proteins; within this range, C=O stretching vibrations of the peptide bond is also observed. The C-H bending vibrations of the CH_3, CH_2, and CH groups were also visible between 1500 and 1200 cm^{-1}, whereas the carbohydrate fingerprint bands were seen between 1200 cm^{-1} and 900 cm^{-1} [32, 33]. Increased intensities of these groups suggested the presence of protein and lipids useful for germination and growth. At the same time, the plasma generated active species and ions also oxidised the surface and enhanced the seed's wettability, stimulated water uptake and improved the seed germination [34].

Fig. 6 The difference between the morphology of untreated and Ar plasma-treated seeds



Conclusions

In this study, a plasma jet was designed in a jet-to-jet coupling and quadruple configuration based on the principle used in DBD, as shown in Fig. 1, to ensure uniform but simultaneous treatment over a large surface area. The plasma properties of this setup, such as electron temperature and density, were measured with OES and computed to be 1.4487 eV and 3.4867×10^{15} cm^{-3}, respectively.

To the best of our knowledge, this study also demonstrated for the first time, the chilli seeds exposed to atmospheric-pressure Ar plasma ion, resulting in significant germination and growth improvement. Ar plasma-treated chilli seeds demonstrated significantly higher growth factors (root potential, shoot length, germination potential and germination percentage) than those of untreated seeds. In essence, plasma-treatment induced chemical and physical changes, such as surface etching and surface functionalisation, to hydrophilize the seed's surface and enhance water absorption. The EC and water absorption values, which raised significantly to enable quicker nutrient transfer and the provision of chemical energy

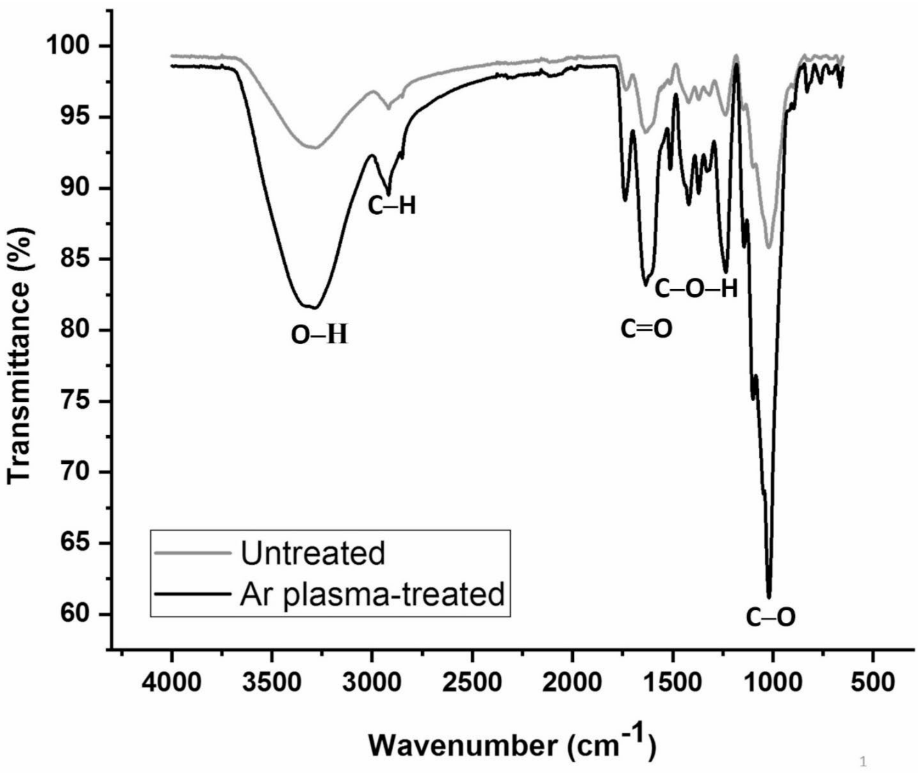


Fig. 7 FTIR spectra of untreated and Ar plasma-treated chilli seeds

to initiate germination, served as evidence for this mechanism. SEM and FTIR measurements confirmed the surface etching and chemical functionalisation of the seeds. Our next study will be focused on elucidating the effect of plasma-treatment at the genetic level of the chilli seeds.

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Data Availability Not applicable.

Code Availability Not applicable.

Declarations

Conflict of Interest Not applicable.

Competing Interests The authors declare no competing interests.

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