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LOW-PRESSURE RF DIELECTRIC BARRIER DISCHARGE PLASMA TREATMENT OF 'CARRIZO' CITRANGE SEEDS

TRATAMENTO DE PLASMA DE DESCARGA DE BARREIRA DIELÉTRICA DE RF DE BAIXA PRESSÃO DE SEMENTES DE CITRANGE 'CARRIZO'

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ABSTRACT

Seed quality is crucial for the development of vigorous plants, and 'Carrizo' citrange is widely used as a rootstock in citrus farming. However, factors such as storage and temperature affect germination; therefore, sustainable treatment alternatives are being explored, including the use of cold plasma. This study evaluated the effects of cold plasma treatment on the germination of 'Carrizo' seeds. A low-pressure radio-frequency dielectric barrier discharge reactor with nitrogen gas was used to treat the seeds for 1, 2, and 3 minutes. An increased wettability was observed for the treated samples. Fourier-transform infrared and X-ray photoelectron spectroscopies revealed chemical modifications on the surface, with an increase in oxygenated compounds. Scanning electron microscopy showed surface erosion. The treatment reduced the germination rate and did not affect shoot or root growth, with a prevalence of polyembryonic seedlings.
Keywords: Surface modification; Polyembryony; DBD plasma treatment.

RESUMO

A qualidade das sementes é crucial para o desenvolvimento de plantas vigorosas, e a citrange 'Carrizo' é amplamente utilizada como porta-enxerto na citricultura. No entanto, fatores como armazenamento e temperatura afetam a germinação; portanto, alternativas sustentáveis de tratamento estão sendo exploradas, incluindo o uso de plasma frio. Este estudo avaliou os efeitos do tratamento com plasma frio na germinação de sementes 'Carrizo'. Um reator de descarga de barreira dielétrica de radiofrequência de baixa pressão com gás nitrogênio foi usado para tratar as sementes por 1, 2 e 3 minutos. Um aumento na molhabilidade foi observado para as amostras tratadas. Espectroscopias de infravermelho com transformada de Fourier e fotoelétrons de raios X revelaram modificações químicas na superfície, com aumento de compostos oxigenados. A microscopia eletrônica de varredura mostrou erosão da superfície. O tratamento reduziu a taxa de germinação e não afetou o crescimento da parte aérea ou da raiz, com prevalência de plântulas poliembriônicas.
Palavras-chave: Modificação de superfície; Poliembrionia; Tratamento com plasma DBD.

1 INTRODUCTION

Seed quality is crucial for the success of a vegetable crop. It must exhibit high rates of vigor, germination, and health, in addition to ensuring physical and varietal (genetic) purity. Using high-quality seeds produces high-performance plants that are strong, vigorous, and adaptable to different climatic conditions (KOLCHINSKI et al., 2005; KRZYZANOWSKI, 2018; SPADOTTI et al., 2008).

Rootstock and scion combinations are widely used in citrus farming to improve productivity, fruit quality, and increase tolerance to biotic (diseases and pests) and abiotic stresses (CHAHAL et al., 2018; OUSTRIC et al., 2017). Among citrus species, the ‘Carrizo’ citrange [Poncirus trifoliata (L.) Raf. × Citrus sinensis (L.) Osbeck] stands out for its global importance due to its tolerance to citrus tristeza virus (CTV), root rot caused by Phytophthora spp., and root-knot nematodes. This rootstock performs well in heavier clay soils and is compatible with most citrus varieties (CHAHAL et al., 2018; SHEHATA e AHMED, 2023).

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'Carrizo' citrange rootstocks are propagated by seed, which is readily available, inexpensive, abundant, and faithfully growing. However, factors such as storage in cool, dry conditions, seed size and maturity, source fruit size, seed coat removal before storage, and temperature influence seedling germination and growth (CHAHAL et al., 2018; NAVARRO-GOCHICOA et al., 2024).

Another important factor for citrus rootstock cultivation is polyembryony, which allows clonal propagation, ensuring uniformity and plants faithful to the original type. It is is beneficial for maintaining desirable characteristics in commercial citrus production (MARTÍNEZ-OCHOA et al., 2022; WOO et al., 2019).

Pre-planting seed treatment is an economically advantageous practice, as it can increase germination and cultivar vigor, providing a greater financial return for the producer. The most commonly used inputs include chemicals/pesticides such as fungicides, herbicides, and other agrochemicals, or biological agents, which are generally labor-intensive and expensive. Even more problematic is the fact that residual chemicals on the seed surface can contribute to soil pollution, harming the environment and often the health of consumers (AVELAR et al., 2011; WASKOW et al., 2021).

Thus, the search for more environmentally friendly technologies has been the subject of research in this field, such as physical methods such as ultrasonics, electric field treatment, magnetic field treatment, and ion beam cavitation, among others. These methods, although effective in increasing plant vigor, can damage seed cells, increasing the number of destroyed seeds, and potentially resulting in uneven treatment (AVELAR et al., 2011; GUO et al., 2018). The ideal solution for seed treatment must meet the following characteristics: fast and uniform germination; energy efficient; economically viable; does not leave toxic residues; does not cause damage to the material; preserves its cellular integrity and favors storage (GUO et al., 2018; PASSOS, Alexandre Martins Abdão Dos et al., 2023).

In recent years, the use of cold plasma for seed treatment has been investigated (LI et al., 2016; MOHAJER et al., 2024; MOLINA et al., 2018a, b; PEREA-BRENES et al., 2025; RANDENIYA e DE GROOT, 2015). Some plasma processes have been shown to improve germination, promoting more vigorous plant growth and increasing crop yield. These benefits stem from changes in wettability and chemical compatibility, resulting in increased germination rates and biomass. The

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treatment also strengthens plant resistance to diseases by reducing the levels of microbial pathogens and insects, in addition to improving tolerance to climatic stress and reducing water consumption. Plasma processes also minimize the generation of detectable toxic waste, minimizing environmental impact, have low energy consumption, affordable cost, and high efficiency, requiring small amounts of raw materials and inputs to carry out seed treatment (MAZUR et al., 2023; PASSOS, Alexandre Martins Abdão Dos et al., 2023; PÉREZ-PIZÁ et al., 2020; PUAČ et al., 2018; WASKOW et al., 2021).

The use of plasma as a surface treatment technique in biology is most commonly achieved through dielectric barrier discharges (DBDs) and atmospheric pressure plasma jets (APPJs). Current studies demonstrate that plasma seed treatment is feasible and can be applied to major crops such as soybeans, wheat, and others. Ling and colleagues (2018) observed positive effects on seed germination and growth of soybean seedlings treated with helium gas plasma generated by a radiofrequency (RF) source (13.56 MHz) with power ranging from 60 to 120 W for 15s (LI et al., 2016). They observed that the treatment performed with 80 W power had the greatest stimulating effect, increasing germination rates, vigor, contact angle, and decreasing water absorption (LING et al., 2014). Pérez-Pizá et al. (2020) also studied soybean seeds, evaluating the treatment with plasma generated through a DBD, operating at atmospheric pressure, with superimposed flows of O2 or N2 (flow rate of 6 NL min −1), with a voltage of 25 kV and frequency of 50 Hz, with seed exposures to the plasma for 2 and 3 minutes. They observed a 1.6-fold increase in nitrogenase activity in plasma-treated samples (PÉREZ-PIZÁ et al., 2020). Guo and collaborators (2018) evaluated the improvement in wheat seed vitality after DBD plasma treatment at atmospheric pressure and room temperature, a treatment time of 4 min, and a power supply frequency of 50 Hz, with varying discharge voltages. They observed positive effects on germination and seedling growth vigor after treatment at 11.0 kV (GUO et al., 2018).

Molina et al. (2018) proposed treating different types of seeds with plasma to obtain a hydroabsorbent surface, using helium gas and atmospheric air as the plasma gas, with a frequency of 16 kHz, voltage of up to 20 kV, and exposure times ranging from 10 s to 300 s. They observed that samples treated for short times (<30 s) showed more efficient germination, while plasma treatments for longer times (up to 300 s) impaired germination (MOLINA et al., 2018a). This same

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trend was observed by Ouf and Almarashi (2022), who evaluated the action of atmospheric plasma on Moringa oleifera (white acacia) seedling seeds and observed that low plasma doses (2.46 and 4.35 mJ) induced an increase in seed germination, a significant increase in chlorophyll content, leaf area, and antioxidant activities. Treatment using plasma generated with higher doses (≥ 10.94 mJ) was harmful to the seeds (OUF e ALMARASHI, 2022).

In addition to large crops, studies have been carried out with seeds of perennial crops such as moringa and carob (BEN OTHMAN et al., 2024; OUF e ALMARASHI, 2022). The treatment of fruit seeds such as grapes, tomatoes, and melons has shown positive and promising results (LIU et al., 2019; SEDDIGHINIA et al., 2020; TOUNEKTI et al., 2020). However, we have not found any studies with plasma in citrus seeds in the literature.

Given the growing demand for clean, economical and sustainable technologies to improve seed germination and, consequently, plant growth, the objective of this study is to evaluate the effects of treating citrus rootstock seeds, citrange 'Carrizo', using low-pressure RF DBD plasma, considering that the modification of the seed surface by non-thermal plasma is a fast, economical and environmentally friendly technology, in addition to not finding works in the literature on citrus seed treatments using plasma.

2 MATERIALS AND METHODS

2.1 Seed Treatment with Plasma

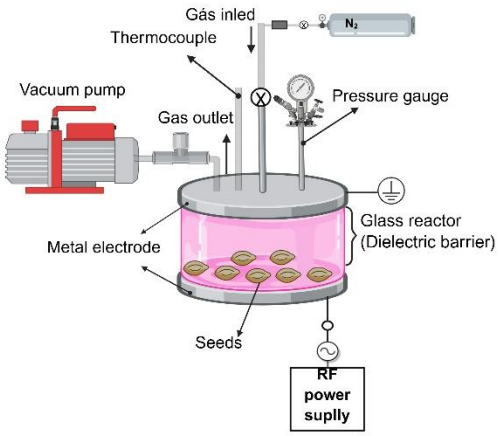
'Carrizo' citrange seeds were carefully removed from the fruit and washed with running water. The seeds were treated with plasma generated through a DBD at low pressure (1.5 mbar). The reactor consists of a glass vessel andof two parallel electrodes, one polarized (bottom) and the other grounded (top), spaced 20 mm apart by the glass itself. The glass covers the driven electrode, acting as a dielectric barrier and sample holder. Nitrogen (N2) was used as the treatment gas at a flow rate of 90.1 sccm, aiming to introduce oxygen- and nitrogen-containing groups to the seed surface, promoting their oxidation and the formation of reactive oxygen and nitrogen species, which contribute to increased hydrophilicity and water absorption capacity. A power of 30 W was used using a RF

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power source (13.56 MHz), as can be seen in Figure 1. Plasma treatment was conducted on the seeds for 1, 2, and 3 minutes with temperatures ranging from 25 to 35°C.

Figure 1 - (A) Schematic of the experimental setup used to treat 'Carrizo' citrange seeds with nitrogen plasma; (B) Photograph of the reactor under operating conditions



(A)



(B)

Source: Authors, 2025.

2.2 Water Contact Angle (WCA)

Mobility measurements of plasma-treated and untreated seeds were performed using a Ramé-Hart goniometer, model 590, equipped with the DROPimage Advanced high-resolution image processing system. The tests were conducted at room temperature (25 ± 2°C) using 2.0 µL drops of deionized water. Contact angle measurements were performed in triplicate, so the results presented are an average of these measurements with their respective standard deviations.

2.3 Scanning Electron Microscopy (SEM)

The morphology of plasma-treated and untreated seeds was evaluated using scanning electron microscopy (SEM) (JEOL microscope, model JSM-6701F). The seed samples were

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embedded in copper stubs using double-sided carbon tape and metallized with a thin layer of gold (Au) using a vacuum device (Bal-Tec model SCD 050, Balzers, Liechtenstein).

2.4 Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra for plasma-treated and untreated seeds were obtained using a Bruker Invenio-S (Akbou, Algeria). Each spectrum was recorded with scans and a resolution of 4 cm^{-1}, covering the spectral range from 4000 to 800 cm^{-1}. Data were collected using the ATR (Attenuated Total Reflection) technique.

2.5 X-Ray Photoelectron Spectroscopy (XPS)

X-Ray photoelectron spectrocopy (XPS) spectra of plasma-treated and untreated seeds were obtained using a ThermoFisher Scientific K-Alpha spectrometer operating in constant pass energy mode and using a monochromatic Al Kα source (1486.6 eV). The spectra are an average of 10 scans (X-ray beam spot diameter 400 µm). Survey spectra were obtained at a pass energy of 200.00 eV, a dwell time of 10 ms, and an energy step of 1.00 eV. High-resolution C 1s spectra (279.00–298.00 eV) were obtained using a pass energy of 20.00 eV, a dwell time of 100 ms, and an energy step of 0.025 eV. The bond energy scale was referenced to the C-C/C-H bonds at 284.8 eV. Fits were performed using Gaussian-Lorentzian convolution (30% Lorentzian). XPS spectra were analyzed using Avantage software, using the Shirley method, with the Smart option of the software, to remove the background signal caused by inelastic collisions.

2.6 Germination Percentage, Polyembryony Rate, and Seedling Growth

Twenty untreated seeds were used as a control, while 20 seeds were used for each plasma treatment duration (1, 2, and 3 min). For each experimental condition, all 20 seeds were placed in the reactor simultaneously and subjected to a single plasma treatment cycle. Thus, the plasma treatment was applied to the group of 20 seeds as a whole, whereas individual seeds were evaluated in the subsequent germination experiment. There was no supplemental light or nutrients. Sowing was done in tubes containing a commercial substrate, Tecnomax, composed of pine bark, vermiculite, carbonized rice hulls, and NPK additive. Sixty-five days after the start of the germination

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process, the number of germinated seedlings was calculated to determine the germination percentage, the number of germinated seedlings per seed to determine polyembryony, and the aerial part and root system were measured to assess growth. The results are expressed as means with their respective standard deviations.

2.7 Water Absorption

The seeds were immersed in a 100 mL beaker containing distilled water and then kept in a chamber at 25° C with constant lighting, according the methodology described by Silva et al. (2017). The level of absorption was assessed at intervals of 0, 4, 12, 20, and 38 hours. At the end of each interval, the samples were removed from the beaker, dried superficially with absorbent paper, and weighed. The water absorption at each time point was calculated using the following equation:

$$\%\ \text{water absorption} = \frac{(\text{Wf} - \text{Wi})}{\text{Wi}} x\ 100$$

Where, Wi: initial weight of seeds; Wf: final weight of seeds at each time.

2.8 Statistical analysis

The data were subjected to a one-way analysis of variance (ANOVA). Means were compared using Tukey's test at a significance level of 5% ($p < 0.05$). All statistical analyses were performed using Statistica® software, version 10.0 (StatSoft Inc., Tulsa, OK, USA).

3 RESULTS AND DISCUSSION

3.1 Water Contact Angle (WCA)

Seed surface contact angle was determined for control samples (untreated) and those treated with plasma for 1, 2, and 3 minutes. Measurements were taken immediately after treatment (1 day) and for the following 10 days, as shown in Table 1. A surface's interaction with water is categorized based on the observed contact angle between the surface and a water droplet. This classification is defined as complete hydrophilicity ($\theta \leq 60°$), moderate hydrophilicity ($60° < \theta < 90°$), and

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hydrophobicity (θ ≥ 90°), where θ represents the contact angle(KANG et al., 2020). Taking these parameters into account, the results observed in this study reveal that plasma treatment significantly decreased the contact angle of treated seeds compared to the control. For all samples, immediately after treatment, hydrophilicity showed no statistical difference between the 1, 2, and 3 minute treatments. However, after aging for 10 days, with the seeds kept refrigerated (6 ± 2)° C, the samples treated for 1 min and 2 min showed hydrophobic recovery, with contact angle values similar to the control sample. Thus, the 3-minute treatment presented the best results regarding the change in seed hydrophilicity. The increase in hydrophilicity is an important factor, since the literature reports a relationship between seed water affinity and a potential improvement in germination (TOMEKOVÁ et al., 2024; WANG et al., 2017).

Table 1 - Water contact angle (WCA) of untreated 'Carrizo' citrange seeds (control) and after nitrogen plasma treatment, treatment times of 1, 2, and 3 min, with 30 W power.

Sample	CA_1d	CA_2d	CA_3d	CA_7d	CA_10d
Control	$80.7\pm0.8^{a,A}$	$80.6\pm2.3^{a,A}$	$80.7\pm4.4^{a,A}$	$84.2\pm3.1^{a,A}$	$80.2\pm5.0^{a,A}$
1 min.	$52.8\pm5.8^{b,A}$	$68.1\pm4.7^{b,B}$	$75.3\pm1.7^{b,B}$	$75.2\pm7.7^{b,B}$	$75.4\pm4.3^{a,b,B}$
2 min.	$55.3\pm2.4^{b,A}$	$59.1\pm3.9^{c,A}$	$64.9\pm2.9^{c,B}$	$68.5\pm4.1^{c,B}$	$69.8\pm4.0^{b,B}$
3 min.	$50.0\pm6.5^{b,A}$	$54.9\pm1.7^{c,A,B}$	$54.3\pm1.6^{d,A,B}$	$56.2\pm1.9^{d,A,B}$	$58.1\pm8.0^{c,B}$

Values presented as mean ± standard deviation. Different lowercase letters in the same column indicate a significant difference in treatment time; different uppercase letters in the same row indicate a significant difference in storage time ($p < 0.05$) when analyzed by Tukey's test.

Increased hydrophilicity is related to chemical and physical mechanisms of plasma action on the seed surface, such as the degeneration or degradation of physicochemical structures, such as cellulose, the removal of lipid groups, and the corrosion of the seed surface. What can also occur is the functionalization of the surface with reactive nitrogen species formed in the plasma (atomic nitrogen "N", in excited states N* and N2*, ionized N+ and N2+), which react on the surface and form reactive oxygen and nitrogen species (RONS), contributing to increased wettability. This facilitates wetting and water permeation, improving the germination process (GURAGAIN et al., 2024; HOLUBOVÁ et al., 2021; KANG et al., 2020; TOMEKOVÁ et al., 2024; WANG et al., 2017).

The effects of plasma depend on both the type of treatment applied and the characteristics of the treated species, as different species have distinct morphologies (MOTRESCU et al., 2023).

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However, other studies have reported a decrease in the water contact angle in different seeds after plasma treatment, such as: rice seeds (KANG et al., 2020), nasturtium (MOLINA et al., 2018a), lentils, beans, and wheat(BORMASHENKO et al., 2012), wheat (VELICHKO et al., 2019), mimosa (JUNIOR et al., 2016), wheat and barley (HOPPANOVÁ et al., 2020), soybeans (TOMEKOVÁ et al., 2024), cotton (WANG et al., 2017), broccoli and watercress (MOTRESCU et al., 2023), rice (RONGSANGCHAICHAREAN et al., 2022), beans (RECEK et al., 2021), fenugreek (GURAGAIN et al., 2024).

Another characteristic observed for the contact angle is hydrophobic recovery, that is, the gradual return of the contact angle to more hydrophobic values after some aging time (FRIDMAN, 2008; RECEK et al., 2021). In the present work, samples treated for 1 min, 2 min, and 3 min showed hydrophobic recovery of 93%, 80%, and 67%, respectively, after three days of aging. The 3-minute treatment, however, preserved its initial characteristics. A longer treatment time results in more stable hydrophilicity over time; this trend is described in the literature for seeds and other polymers (BORMASHENKO et al., 2012; FRIDMAN, 2008; RECEK et al., 2021; VESEL et al., 2021). However, the reason for this phenomenon is not yet well defined. One explanation is that prolonged treatment results in a surface with a higher concentration of functional groups and, consequently, more stable in terms of affinity with water (RECEK et al., 2021; VESEL et al., 2021).

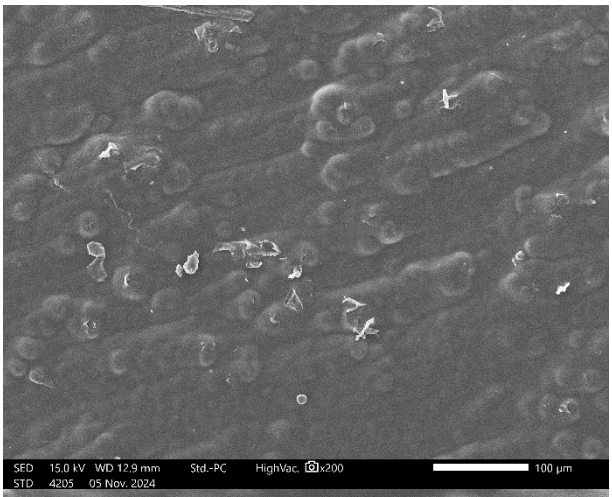
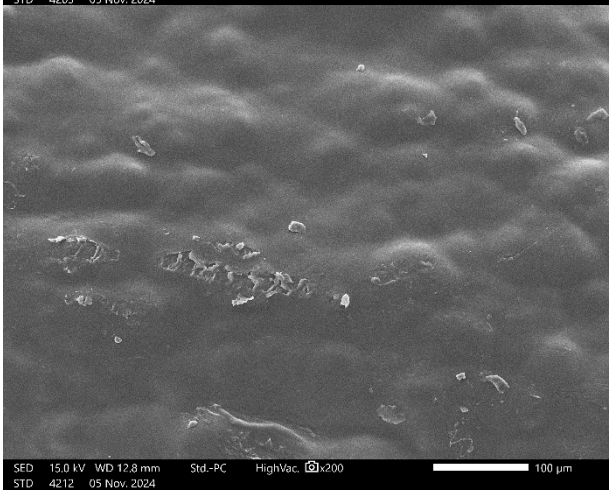
3.2 Scanning Electron Microscopy (SEM)

The surface morphology of 'Carrizo' citrange seeds was analyzed by SEM (Figure 2).

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Figure 2 - Micrographs obtained by scanning electron microscopy (SEM) of untreated 'Carrizo' citrange seeds (control) and after treatment with nitrogen plasma, treatment time of 1, 2, and 3 min, with 30 W of power.

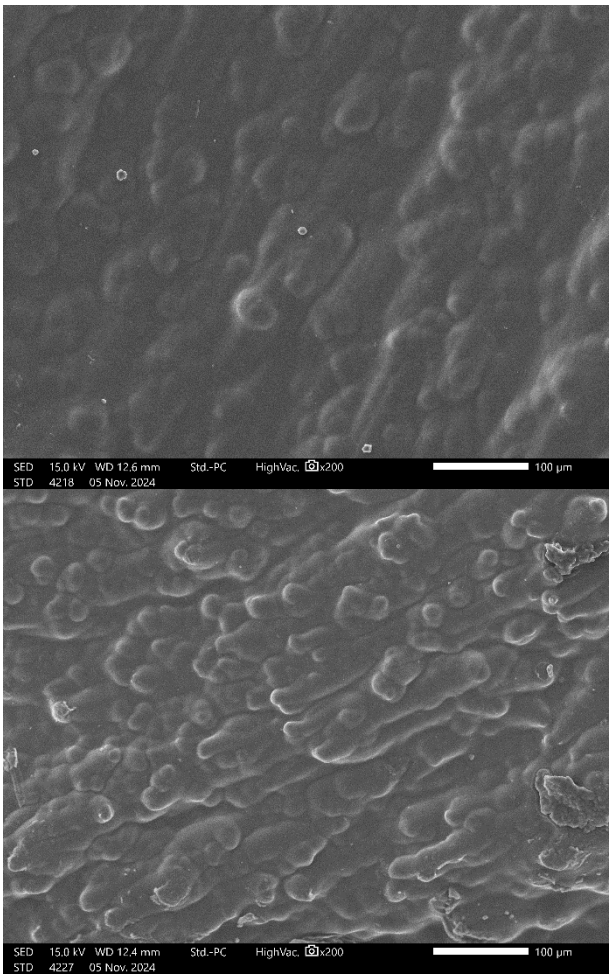
Sample	**Micrography**
Control	
1 min	

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2 min

3 min



Source: Authors, 2025.

Plasma treatments lasting 1, 2, and 3 min did not appear to cause visible damage to the outer surface of the seeds, as no cracks or cracks were observed. However, the difference in surface characteristics between the treated seeds is evident, especially those subjected to the 3-min treatment, which present a more striated surface compared to the control. Similar results were reported by Garagain et al. (2024) in fenugreek seeds subjected to plasma, attributing these changes to the effect of plasma erosion, which removes surface materials and results in increased roughness and microstructuring of the outer layers of the seed coat (GURAGAIN et al., 2024; RECEK et al., 2021). However, seeds are biological materials that naturally present surface variations, which can

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influence the pattern observed in the micrographs. Therefore, it is essential to interpret seed images obtained by SEM with caution (WASKOW et al., 2021).

Although SEM analysis provides useful information on seed surface morphology, its application is limited to fully explaining the effects of plasma treatment, as it is still unclear whether the cracks that plasma can cause in seeds are essential for improving plant growth (WASKOW et al., 2021). Furthermore, other authors observed no changes in cracks or perforations in seed morphology after plasma treatment, but revealed improvements in water affinity and germination of different seed types (BORMASHENKO et al., 2012; MOLINA et al., 2018b; RUSU et al., 2018; ŠTĚPÁNOVÁ et al., 2018). Therefore, it is essential to complement morphological analysis with analytical methods such as ATR-FTIR and XPS, which were performed later in this work.

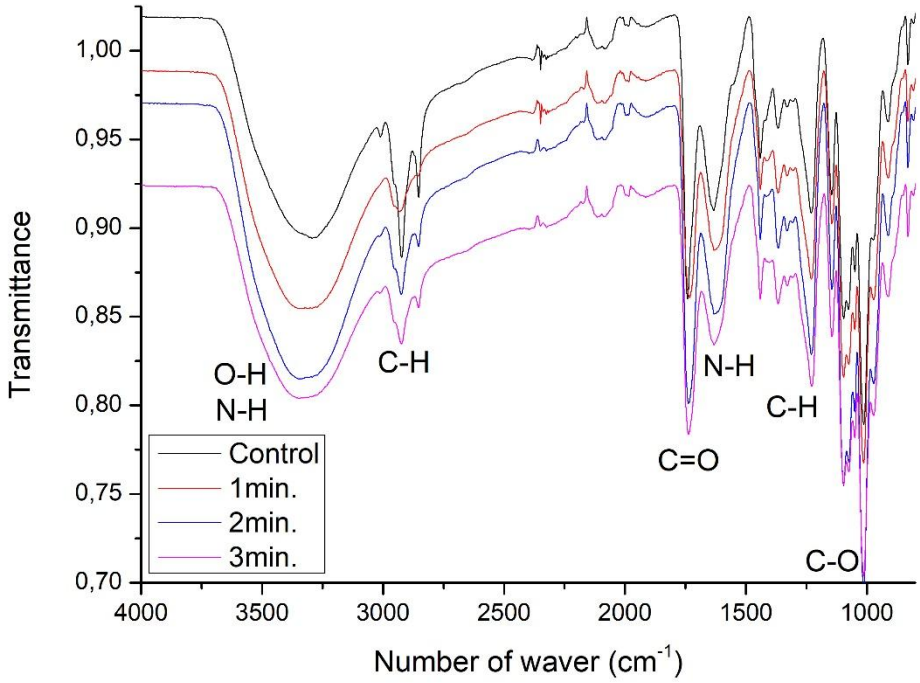
3.3 Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR)

To determine the effect of plasma on seed surface chemical properties, ATR-FTIR analysis was performed on untreated 'Carrizo' citrange seeds (control) and treated with DBD plasma under low-pressure nitrogen at 30 W for 1, 2, and 3 min. The ATR-FTIR spectra (Figure 3) revealed the presence of components characteristic of the seed coat, such as cellulose, which is hydrophilic and insoluble in water, and wax, which is hydrophobic and responsible for this behavior in untreated seeds (TOMEKOVÁ et al., 2024; WANG et al., 2017).

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Figure 3 - Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) of untreated 'Carrizo' citrange seeds (control) and after nitrogen plasma treatment, treatment time of 1, 2 and 3 min, with 30 W power



It is possible to observe characteristic bands of polysaccharides such as cellulose (1200–800 cm−1) with glycosidic bonds and chemical groups, C–O, C–C, C–OH, lipids (3000–2800 cm−1 and 1820–1760 cm−1) represented by C–H and C=O bonds, respectively, which indicated the presence of wax, cutin and oil, N–H group (1580–1495 cm−1 and 3500–3070 cm−1) attributed to the structure of proteins (MOLINA et al., 2018b; TOMEKOVÁ et al., 2024; WANG et al., 2017). The band in the 3500–3000 cm−1 region corresponds to the O–H stretching vibrations originating from water (MOLINA et al., 2018a; RUSU et al., 2018; TOMEKOVÁ et al., 2024) . No significant differences were observed in the shape of the spectra of the treated samples compared to the untreated ones, indicating the destruction or modification of the chemical bonds belonging to the components that form the external part of the seed (MOLINA et al., 2018b; TOMEKOVÁ et al., 2024). On the other

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hand, the treatment modified the intensity of the bands, which decreased with the application of the N2 plasma, but did not affect their positions (MOLINA et al., 2018a; WANG et al., 2017). This trend was observed mainly in bands characteristic of waxes and lipids C–H (3000–2800 cm−1) and C=O (1460–1400 cm−1). This fact, together with the degradation of cellulose due to the exposure of the seed to plasma, may justify the modification of the contact surface with water, corroborating the results observed for the contact angle analysis (MOLINA et al., 2018a, 2020; WANG et al., 2017).

The difference in O–H band intensity may be related to seed water loss and mass loss after plasma treatment, mainly due to water removal by the reactor's low-pressure vacuum system (Table 2) (MOLINA et al., 2018b).

Table 2 - Mass of untreated 'Carrizo' citrange seeds (control) and after nitrogen plasma treatment, treatment times of 1, 2, and 3 minutes, with 30 W power.

Sample	Seed mass (g)	Mass loss (g)
Control	0.31±0.02[a]	-
1 min	0.24±0.04[b]	0.07
2 min	0.24±0.03[b]	0.07
3 min	0.24±0.03[b]	0.07

Values presented as mean ± standard deviation. Different letters in the same column indicate a significant difference ($p < 0.05$) when analyzed by Tukey's test.

3.4 XPS

For the XPS results, survey spectra of the seeds surface were performed, and the elements analyzed were carbon and oxygen, with nitrogen, silicon, and calcium also observed. Table 3 shows a clear decrease in carbon content and an increase in oxygen in treated seeds compared to the control. Other authors have observed this same trend and the presence of some of these compounds in different seed matrices (GÓMEZ-RAMÍREZ et al., 2017; GURAGAIN et al., 2024; MOLINA et al., 2018b, 2020; PEREA-BRENES et al., 2022; ŠTĚPÁNOVÁ et al., 2018; WASKOW et al., 2021).

The XPS analysis data can be correlated with the results observed in the FTIR spectra. Table 3 and Figure 4 of the XPS clearly show a decrease in the atomic percentage of carbon (between 4 and 17%), mainly in the C-C/C–H bond, and an increase in oxygen (between 5 and 14%), with O=C–

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O, C=O, and C–O bonds. In the FTIR spectrum (Figure), the decrease in the C–H bands (3000–2800 cm−1 and 1820–1760 cm−1) after treatment confirms that carbon oxidation occurred after plasma application, which suggests that the plasma chemically modifies the seed surfaces and, thus, contributes to increased hydrophilicity and water absorption into the seeds (WASKOW et al., 2021).

This chemical modification of the seed surface induced by nitrogen plasma can lead to the formation of several reactive oxygen and nitrogen species (RONS) with polar characteristics, such as carbonyl (C=O), carboxyl (C=O and –OH), hydroxyl (–OH), and amino (–NH2) groups (RONGSANGCHAICHAREAN et al., 2022). The XPS analysis results expressed in Figure 4 and Tables 3 and 4 demonstrate the likely formation of these species on the seed surface, represented by a decrease in chemical groups and carbon atomic percentages, and an increase in oxygen and nitrogen. RONS can have positive or detrimental effects on plant development, as they act at different stages of the seed life cycle, influencing germination rate and germination potential, acting on different signaling pathways, and regulating processes (MOTRESCU et al., 2023; RONGSANGCHAICHAREAN et al., 2022).

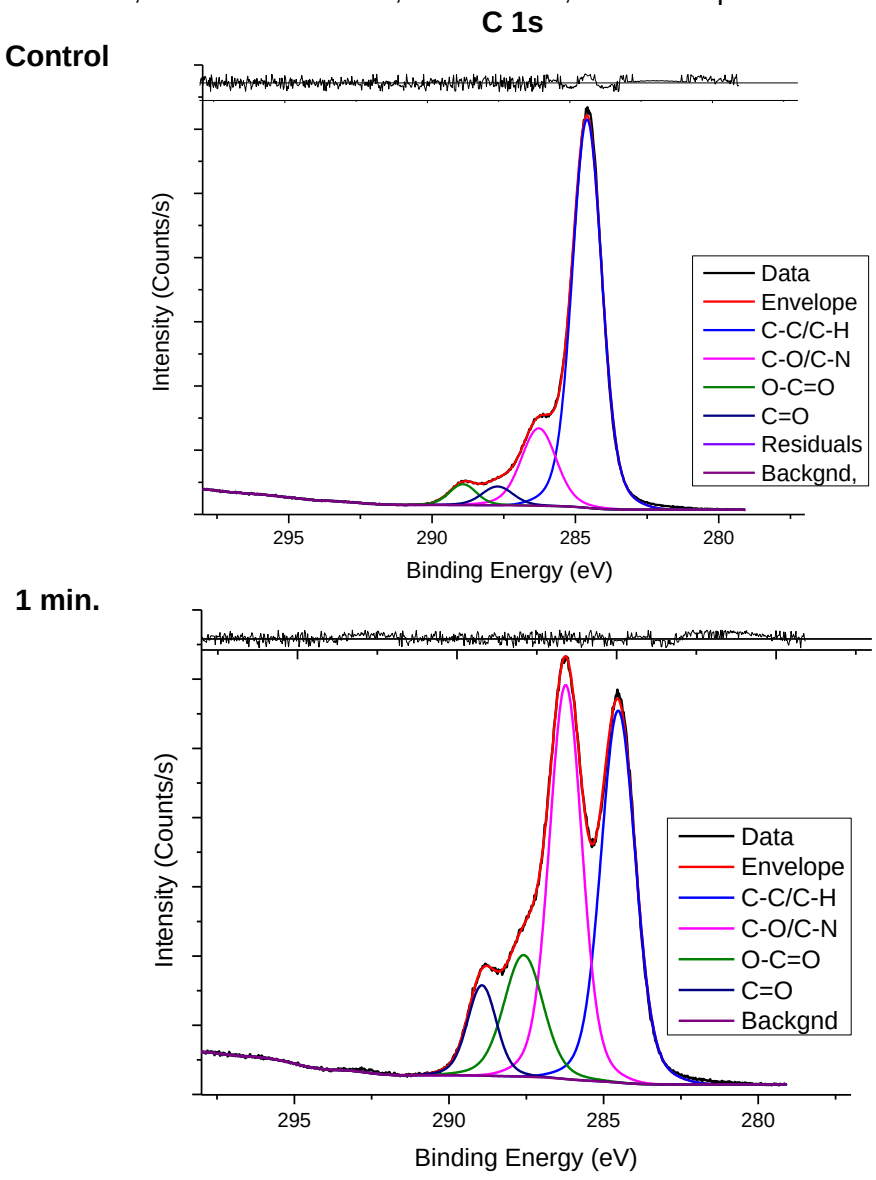
The mechanism of action of RONS involves inducing stress, similar to that caused by drought or salinity. These species promote oxidative and nitrosative stress in exposed cells, triggering the production of other reactive species in response to the initial stress, influencing different stages of plant development and modifying plant physiological characteristics (MOTRESCU et al., 2023).

However, the addition of bound oxygen to the seed surface caused by this oxidation is not stable over time, which may have led to the hydrophobic recovery observed in the water contact angle analysis after storage of the samples for 10 days (GURAGAIN et al., 2024)..

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Figure 4 - X-ray photoelectron spectroscopy (XPS) spectra of untreated 'Carrizo' citrange seeds (control) and after nitrogen plasma treatment, treatment time of 1, 2 and 3 min, with 30 W power.

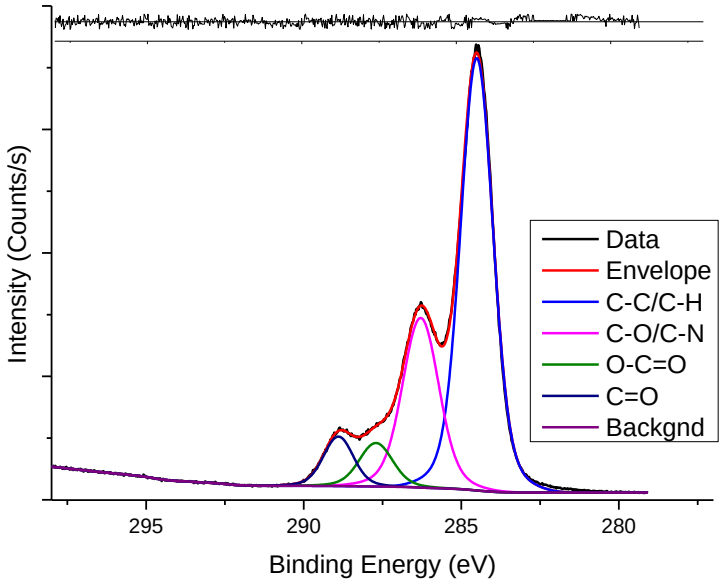


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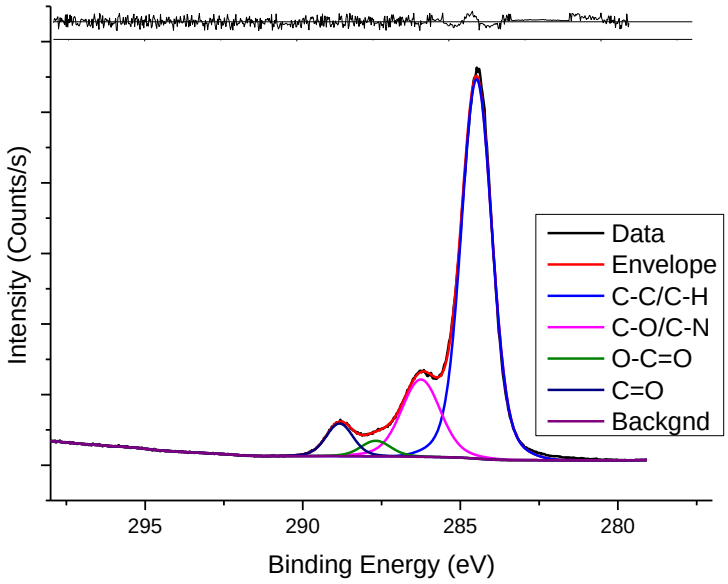
LOW-PRESSURE RF DIELECTRIC BARRIER DISCHARGE PLASMA TREATMENT OF 'CARRIZO' CITRANGE SEEDS

Aline Da Rosa Almeida
Luana Aparecida Castilho Maro
Julio Cesar Sagás
Luis Guilherme Silva Rosa
Marcelo Francisco Krol
Luis César Fontana
Daniela Becker

2 min.



3 min.



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Table 3 - Atomic percentage of carbon, nitrogen, oxygen, silicon, and calcium in untreated 'Carrizo' citrange seeds (control) and after treatment with nitrogen plasma, treatment time of 1, 2, and 3 min., with 30 W of power.

Treatment	Si (at.%)	C (at.%)	Ca (at.%)	N (at.%)	O (at.%)	O/C ratio
Control	1.4	79.7	-	1.5	17.4	0.2
1 min.	0.4	63.4	-	1.4	31.0	0.4
2 min.	1.3	72.7	0.4	1.2	24.4	0.3
3 min.	1.1	76.3	0.4	0.5	21.7	0.3

Table 4 - Results of fitting the C 1s curve of untreated 'Carrizo' citrange seeds (control) and after treatment with nitrogen plasma, treatment time of 1, 2, and 3 min., with 30 W of power

Plasma treatment time	C1s curve fit (%)			
	C-C/C-H 284.8 eV	C-O/ C-N 286.4 eV	C= O 288. 0 eV	O-C=O 289.2 eV
Control	74.2	18.2	4.3	3.2
1 min.	38.3	38.8	14.4	7.9
2 min.	60.7	26.6	6.3	6.4
3 min.	72.8	18.8	2.9	5.5

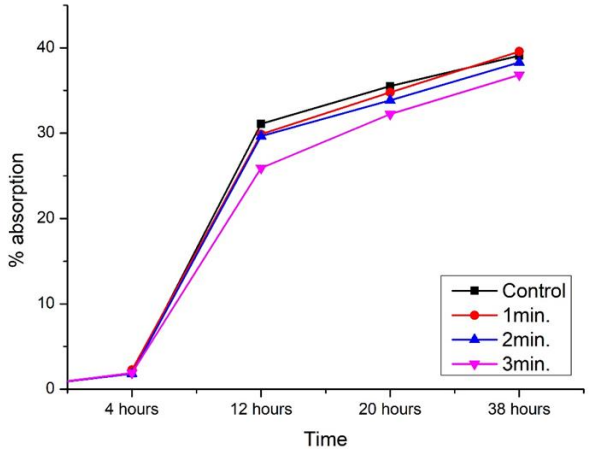
3.4 Water Absorption

The comparison of the water absorption capacity of treated and control (untreated) seeds revealed a very similar trend (Figure 5), suggesting that, although the treatment modifies the surface hydrophilicity and chemical composition of the seed polymeric surface as evidenced by the WCA, FTIR, and XPS tests, it does not affect seed water absorption. This same trend has been observed by other authors. Molina et al. (2020) reported small or insignificant changes in water absorption in plasma-treated wheat seeds compared to untreated ones, despite showing significant variation in surface wettability in WCA analysis and large changes in the chemical composition of the seed surface. Perea-Brenes et al., 2022, who in their work with barley seeds observed similar water absorption in plasma-treated and untreated seeds (MOLINA et al., 2020; PEREA-BRENES et al., 2022).

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Figure 5 - Water absorption in untreated 'Carrizo' citrange seeds (control) and after treatment with nitrogen plasma, treatment time of 1, 2, and 3 min., with 30 W of power.



3.5 Germination Percentage, Seedling Number per Seed, and Aerial and Root Growth

Figure 6 and Table 5 show the final results for the germination test of untreated (control) and treated seeds after 65 days. Overall, no significant differences were observed in aerial and root growth of the seeds after the germination period. Regarding germination percentage, plasma treatment decreased this index. Additionally, a decrease in the overall polyembryony rate was observed in the treated samples. However, in the treated seeds, the prevalence of seedlings with three embryos was higher than in the control (where no seeds with three embryos were observed), which is a desirable characteristic for plants used as citrus rootstock, such as 'Carrizo' citrange. A higher presence of nucellar embryos in seeds increases the probability of generating seedlings of nucellar origin and decreases the probability of developing hybrid seedlings, ensuring the same genetic constitution and resulting in more uniform orchards and vigorous seedlings (MARTÍNEZ-OCHOA et al., 2022; PASSOS, Orlando Sampaio et al., 2006).

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Figure 6 - Image of seedlings germinated from untreated 'Carrizo' citrange seeds (control) and after treatment with nitrogen plasma, treatment time of 1, 2, and 3 min., with 30 W of power.



Table 5 - Germination of untreated 'Carrizo' citrange seeds (control) and after nitrogen plasma treatment, treatment times of 1, 2, and 3 minutes, with 30 W of power.

	Aerial Growth (cm)	Root Growth (cm)	Germination (%)	Polyembryony rate (%)	Seeds with 2 embryos (%)	Seeds with 3 embryos (%)
Control	6.7 ± 0.8^{a}	11.3 ± 0.3^{a}	90	72	100	0
1 min.	6.5 ± 1.1 ^{a,b}	11.1 ± 0.3 ^{a,b}	75	53	75	25
2 min.	5.1 ± 2.3^{b}	9.3 ± 3.6 ^{b}	60	33	75	25
3 min.	6.9 ± 0.8^{a}	11.4 ± 0.5^{a}	80	56	56	44

Values presented as mean ± standard deviation. Different letters in the same column indicate significant difference ($p < 0.05$) when analyzed by Tukey's test.

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4 CONCLUSIONS

Low-pressure RF DBD plasma treatment with nitrogen gas on 'Carrizo' citrange seeds proved effective in modifying their surface, resulting in erosion and an increase in oxygen compounds and a decrease in carbon, resulting in a decreased water contact angle. For germination, plasma treatment reduced this rate; however, a higher percentage of seedlings with three embryos, was observed in seedlings from treated seeds, a desirable characteristic for citrus. Plasma treatment, therefore, showed effects on the surface and germination characteristics of 'Carrizo' citrange seeds.

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