

Dielectric-Barrier Discharge Plasma Effect on the Physico-Chemical Properties of the Seed Coat and Seed Germination of Umbu (*Spondias tuberosa* Arr Camara)

Clodomiro Alves Junior,^{a,*} Mikelly Lima de Farias,^b Jussier de Oliveira Vitoriano,^b Regina Celia de Sousa,^c Marcicleide Lima do Espírito Santo,^c & Salvador Barros Torres^d

^aDepartment of Exact and Natural Sciences, Federal Rural University of Semiarid, Mossoró 59.625-900, Brazil; ^bLabplasma, Federal Rural University of Semiarid, Mossoró 59.625-900, Brazil; ^cDepartment of Physics, Federal University of Maranhão, São Luiz 65080-805, Brazil; ^dDepartment of Plant Science, Federal Rural University of Semiarid, Mossoró 59.625-900, Brazil

*Address all correspondence to: Prof. Clodomiro Alves Junior, Department of Exact and Natural Sciences, Federal Rural University of Semiarid, Mossoró 59.625-900, Brazil, E-mail: clodomiro.jr@ufersa.edu.br

ABSTRACT: *Spondia tuberosa* seeds are enclosed by the walls of a rigid, woody, lignified endocarp. Plasma treatment of such seeds is extremely difficult due to the necessary distance for the plasma species to penetrate until reaching the seed. Several studies have been published describing the success of the plasma treatment in increasing the wettability of seed coats; however, no investigation has also been carried out in situations in which the barrier is not just the integument, but also other anatomical complexities of the seed, such as the walls of the endocarp. In this study, the *S. tuberosa* seed, which is enclosed by an impermeable endocarp, was treated by atmospheric plasma to verify whether the plasma is effective in penetrating to the seed, modifying its germinative properties. Significant changes were observed in the absorption of water leaching substances, germination, and seedling vigor due to plasma treatment. These results show that the plasma, through a mechanism that needs to be better investigated, alter the physical and physiological responses of the seed.

KEY WORDS: atmospheric plasma, dielectric-barrier discharge, germination, dormancy, imbibition

I. INTRODUCTION

The Umbu (*Spondias tuberosa* Arr. Camara), in the Anacardiaceae family, is a native species of the xerophytic biome Caatinga.¹ Under natural conditions, seed germination is slow and non-uniform (12–90 days) ranging from 2% to 26% between 35 and 90 days, respectively.² Throughout the evolutionary process, plants adapted to the semiarid conditions tend to have seed dormancy due to scarcity and irregular rainfall. In the case of *S. tuberosa*, this phenomenon has been reported in the literature and it was found that mechanical dormancy in the endocarp and seed coat hinder water absorption.²

Methods for promoting seed germination are physical (magnetic treatment, sunlight, ultraviolet light, and hot water soaking) and chemical (chemicals, fungicides, and hormones). Although these methods can promote germination to a certain extent, they are

time consuming and labor intensive and produce chemical residues.³ Over the past 15 years, there has been a significant increase in the number of reports of solvent-free, plasma-based techniques to modify 3D porous polymeric materials, with the goal of increasing wettability and preserving the core properties desirable for the intended application.⁴ The non-thermal plasma method has been used in the recent past in a broad spectrum of developmental and physiological processes in plants, including reducing the bacteria-bearing rate of seeds, changing seed coat structures, increasing the permeability of seed coats, and stimulating seed germination and seedling growth.^{5–10} For treatment of the seed coat, plasma is effective at a depth of several nanometers on one side of the substrate and changes the outermost layer of the material without affecting its bulk properties. However, when the seed is naturally enclosed by the endocarp, as is the case with *S. tuberosa*, the treatment gets more complicated.⁸ The external water access from the endocarp to the seed surface occurs through endocarp channels, which are highly complex, porous structures. It is known that plasma penetration depth occurs only in the first monolayer at the solid surface.^{8–9,11} Therefore, seeds that have barriers to plasma penetration are challenging as a technical limit test. Plasma treatment on seeds inside an endocarp, such as the *S. tuberosa* seed, has been prevented or avoided by researchers to date. Literature results have shown that plasma dielectric-barrier discharge (DBD) penetrates a depth of approximately 4 mm in porous materials (textiles).^{12–13} It is known that *S. tuberosa* endocarp has a microphyll structure composed of carbohydrates through which water penetrates and this is the only way that water is absorbed in these seeds.^{14–15}

Based on these factors, there is a possibility that plasma may also permeate endocarp with a similar structure. Observing the germination response, it is possible to estimate the plasma penetration efficiency in seeds such as those of *S. tuberosa*. The purpose of this study was to evaluate the effect of plasma treatment on water absorption, seed germination, and seedling growth when applied to seeds with special anatomy such as that of *S. tuberosa*. Specifically, this study compared plasma-treated and untreated endocarp with the following objectives: (1) to identify the water pathway from endocarp to the seed; (2) to identify the substance leached by the seed during water uptake; and (3) to identify changes in water uptake and germination (radicle emission, rate, and plant quality) after plasma treatment.

II. MATERIALS AND METHODS

A. Plasma Source

The plasma jet was produced from 1 L/min He flow passing through a glass tube of 10 mm diameter and 1 mm thickness containing a cathode ring end (Fig. 1A). A potential of 10 kV and 1 kHz was applied in the ring to generate the DBD produced by a pulsed voltage source (Fig. 1A). The jet was directed to a Petri dish containing the endocarp to be treated. Below the Petri dish, a copper plate was placed and fixed to distribute the plasma evenly and thoroughly, coating the surface of the endocarp. The waveform distance of the plasma jet application was 5 mm, as shown in Fig. 1B.

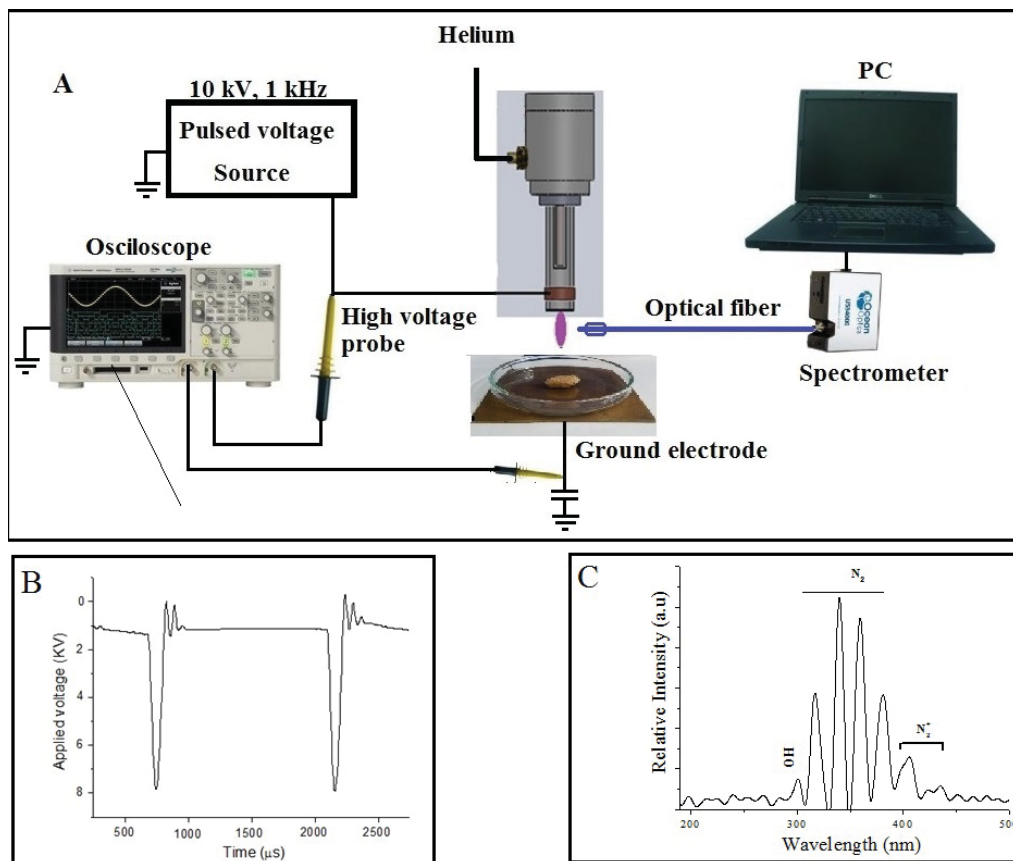


FIG. 1: Plasma source. (A) Experimental apparatus used for plasma treatment of endocarps. (B) Voltage waveforms of the DBD discharge generated. (C) Optical emission spectra of the helium plasma jet below the glass tube.

Plasma optical spectra were obtained with optical fibers positioned laterally to the jet and transmitted to an optical emission spectrometer diagnostic. All treatments were performed within the duration of 1 min and used preset specifications and physical parameters (Table 1). The dissipated electrical power was measured with the aid of lissajous figures monitored on an oscilloscope. A circle 2 mm in diameter was defined as the plasma application area and projected by the jet onto the endocarp.

B. Water Path Identification from the Endocarp Surface to the Seed

Cross-sections and longitudinal sections for the analysis of structure, wettability, and water absorption capacity was performed in different endocarp regions. The structure analysis was performed by light microscopy using toluidine blue dye to highlight the different parts of the endocarp such as the seed, cellulose, and lignified walls. Drops of water were

TABLE 1: Specifications and physical parameters of plasma treatment

Voltage ¹ (kV)	Frequency (Hz)	Energy ² (mJ)	Power ³ (mJ/sec/cm ²)	Gas (units)	Flow (L/min)
10	1 kHz	1.8	5.2	Helium	1

¹Voltage peak to peak.

²Electrical energy dissipated by cycle.

³Mean power per area per second.

placed onto the surface of longitudinal and transverse sections to identify the path of the endocarp seeds. To evaluate the water absorption in the closed endocarps, the individual contributions of two parts called the proximal and distal regions were investigated. Each part was sealed with glue and the water absorbed was quantified and compared with unsealed endocarp. For each experimental condition, five seeds with three repetitions were used.

C. Wettability and Soaking Test

The modification induced by plasma treatment on a surface such as a film is easily measured by the contact angle determination with the sessile drop method. However, in the case of porous materials, the measure is highly affected by the heterogeneous surface structure. Therefore, the porosity of the endocarp determines a suction effect on the water drop and practically prevents contact angle determination. A simple wettability test can be performed on a seed strip kept vertical with the lower end immersed in water–dye liquor.¹⁶ Spontaneous wicking occurs due to capillary forces when the endocarp is immersed with the lower part in the solution (Fig. 2A). Water penetrates into two lower channels, permeating the porous structure of the seed holes to the distal (D) and proximal (P) regions at the top of the endocarp (Fig. 2B).

To test soaking, two groups (untreated and plasma treated) of 28 seeds (seven seeds × four replications) each were immersed into 100 mL of water. At the end of each predetermined interval time, the endocarps were removed from the water and placed on sterile paper to dry the surface.

To calculate the water absorption (immersion), the difference between the initial mass and the final mass was divided by initial mass. The water used for immersion was analyzed with respect to electrical conductivity, pH, and optical absorption of leached substances. The leached substances were analyzed using a spectrophotometer evolution of 600 UV-VIS (EVO600PC model, Thermo).

D. Germination Test

Germination tests were carried out using 30 plasma-treated seeds and 30 untreated seeds and divided into three replicates (groups) of 10 seeds. The seeds were sown in washed and sterilized sand on aluminum trays. Irrigation was provided daily to keep the substratum

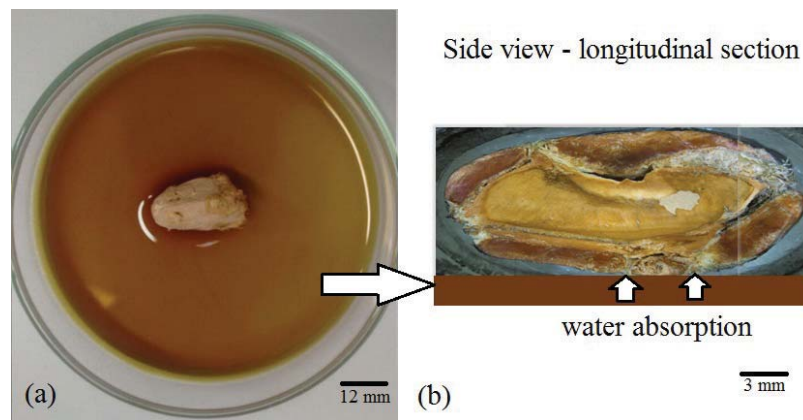


FIG. 2: Capillary rise test used in this experiment. (A) Seed over the water solution of dye. (B) Seed longitudinal section showing the water pathway.

te at 70% of field capacity. The first count was performed after the fifth day of seeding. The experiment was planned with a completely randomized design with four replications. The germination rate (GR), fresh weight (FW), dry weight (DW), moisture (M), and seedling length (SS) were defined as follows: GR (%) = (number of seeds germinated at the last day of germination – 36 days/total number of sown seeds) \times 100%; FW (grams) = total weight of the seedling (root, hypocotyl, and epicotyl) immediately after extraction; DW (grams) = total weight of the seedling after complete dehydration (root, hypocotyl, and epicotyl); M (%) = (mass difference during dehydration) \times 100%; and SS (millimeters) = seedling length (root, hypocotyl, and epicotyl).

E. Statistical Analysis

The statistical analysis for all methods used had an entirely randomized design in factorial-dependent arrangement on each experiment. For the water absorption test in sealed seed parts, one group, 5×3 (number of seeds \times number of repetitions) was used. For other tests such as capillary, soaking, and germination, the factorial arrangement grouping of 7×1 , 7×4 , and 10×3 , respectively, was used. The analysis of variance and comparison of means by Tukey test ($p < 0.05$) was performed using Sisvar® software and the results are presented as mean \pm standard error (SE).

III. RESULTS AND DISCUSSION

A. Endocarp Structure, Composition and Water Path from the Endocarp Surface to the Seed

Cross-sections and longitudinal sections (Fig. 3) made in the endocarp of *S. tuberosa* reveal details of water absorption in key parts. Two different regions should be high-

lighted: the proximal region, which is closer to the stalk of the plant, and the opposite side, the distal region. The holes present on the external surface of the endocarp function as ports from outside to the core for water inlet. Looking at the top of the endocarp, one observes a small hole that was originally trapped in the peduncle (Fig. 3F). In the proximal region, there are two more holes positioned symmetrically with respect to the longitudinal axis of the seed. The distal portion has a large hole called the distal hole (Hd) through which the radicle is emitted. Behind this hole, there are four other holes positioned symmetrically with respect to the longitudinal axis.

The cross-section of the central region (Fig. 3B) shows that there are five lobes containing embryos in which only the highest one is viable to germinate. The longitudinal section (Fig. 3C) performed parallel to the central axis shows the seed encapsulated by the endocarp, which contains some holes that communicate with the seed through channels. These channels consist of a fibrous, porous structure (Fig. 3D, 3G), which draws water by capillary action to the seed. Except for the holes, the endocarp is composed of a dense, hard, impermeable structure consisting of lignified fibers (Fig. 3E). The channels are structured and formed by parallel and transversely distributed fibers, forming pores of different shapes and sizes. This complex and porous structure highly influences the direction of liquid wicking and wetting in the channels. Fiber wettability is a prerequisite for wicking because, if the liquid does not wet the fibers, then it cannot wick into the channel. Wicking can only occur when fibers with capillary spaces in between them are wetted by the liquid. The resultant capillary forces drive the liquid into the capillary spaces.¹⁷

The rate of the seed water absorption is controlled by the crossing time of the seed coat. When water was dripped into the fibrous structure of the channels, they were quickly filled, saturating into the channel interface/seed. To quantify the contribution of each endocarp region, one side was sealed and the absorption curve was determined compared with the unsealed endocarp (Fig. 4). Both sides of the distal portion (red line) and the proximal portion (blue line) were quite similar in values and growing absorption with time. However, compared with the unsealed endocarp, this curve reaches a constant value of absorption. That is, a metabolic process begins in which reservations are converted into simpler compounds for use in germination.¹⁸ During the first 10 hours of soaking, the unsealed endocarp absorbs about 50% of the original mass and maintaining this value until the end of the experiment. Sealed endocarp absorbs about 25% of the initial mass, but continues to increase linearly over time and up to 40% by the end of the experiment. This means that there is a resistance to water absorption probably caused by an increase in the capillary pressure of gases found in the sealed channels. This result also indicates that the complete clearing of the channels prevent imbibition by increasing or impeding the second phase of soaking.

B. Wettability, Water Uptake, and Germination after Plasma Treatment

The wicking time from the bottom to the top of the endocarp was measured (Fig. 5). The time of drag until the Hd is only 13% of the time until the proximal bore. Considering

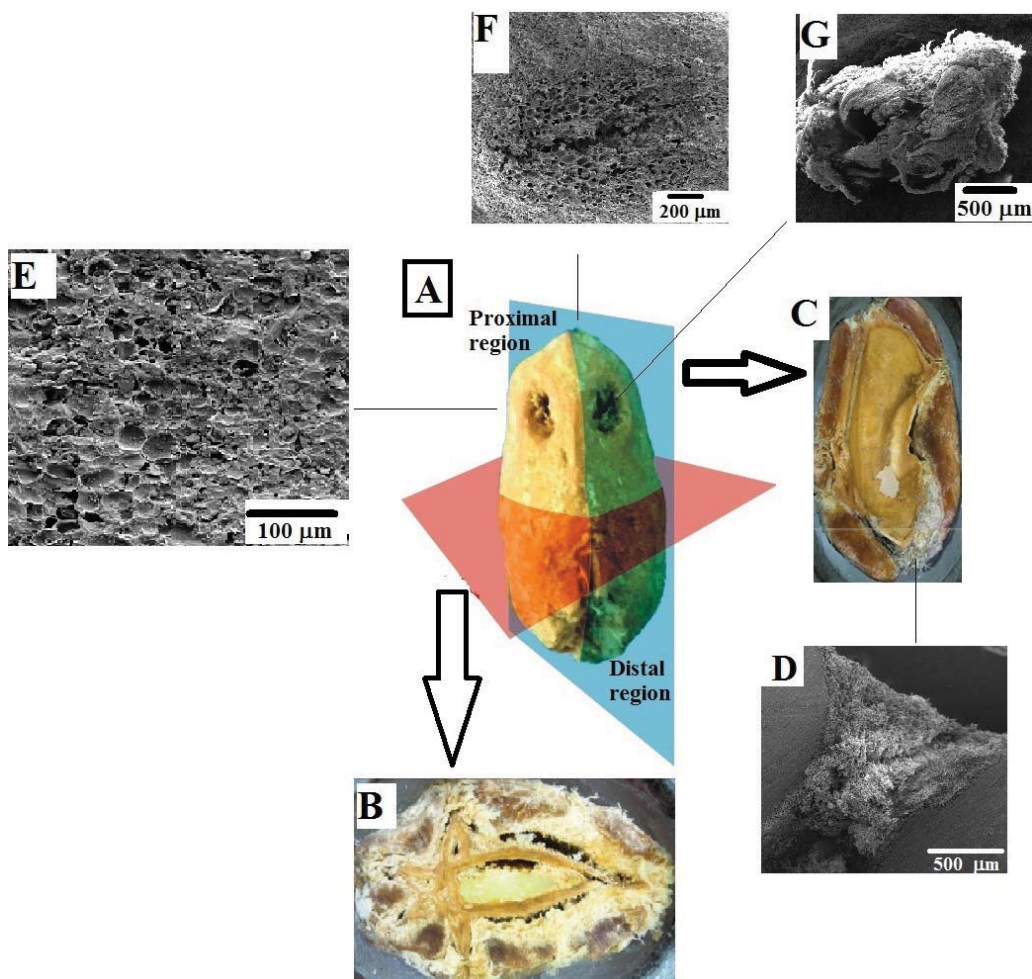


FIG. 3: Details of transverse and longitudinal sections of *S. tuberosa* endocarp. (A) General overview of the endocarp. (B) Cross-section of the central region. (C) Longitudinal section of the central region. (D) Distal channel structure. (E) Endocarp surface of the Hp. (F) Fibrous structure contained in an Hp. (G) Fibrous structure on symmetrical hole.

the path of the liquid to the Hd (Fig. 2B), it appears that the distance is approximately 30% of the traversal distance to the proximal hole (Hp). Therefore, the result obtained indicates greater capillary force and thus a higher rate of water uptake to the Hp. Another explanation for this result is to consider other routes perpendicular to the plane (Fig. 2B).

Comparing the results between treated and untreated samples and taking into consideration the distance to Hd, there is a 28% reduction in rise time after plasma treatment. For the distance to Hp, this reduction was 26%. These results indicate an

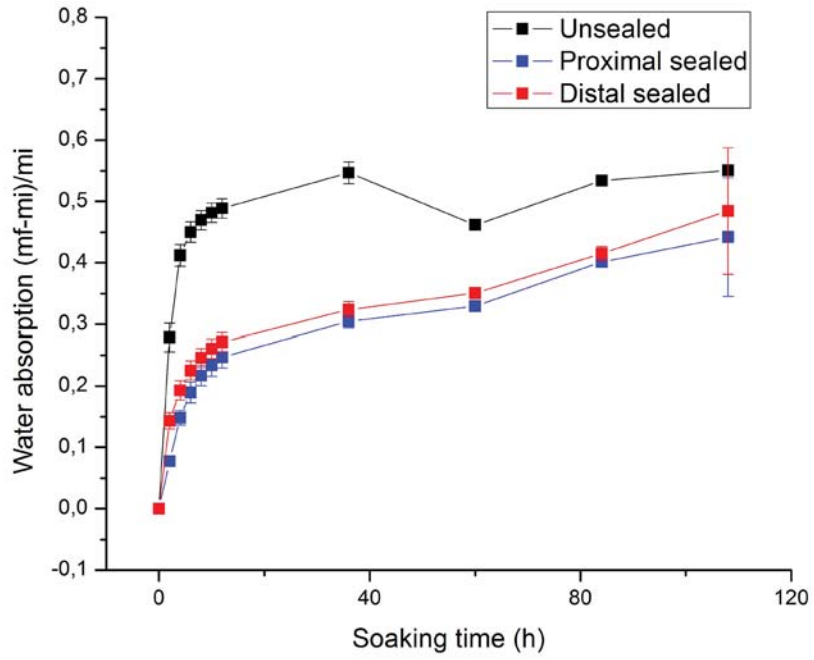


FIG. 4: Absorption of water by the endocarp when the proximal or distal region is sealed

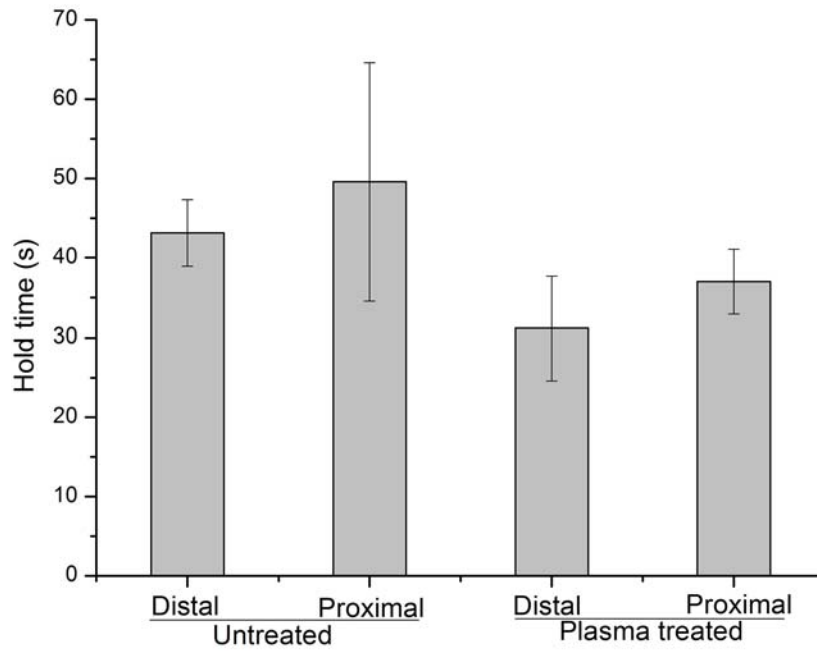


FIG. 5: Wicking time to vertical drag of dye solution from bottom to the Hd or Hp of the endocarp

increase in capillary force caused by the increase in wettability. The positive result of the increased capillary force in plasma-treated endocarps is enhanced by the immersion test in water (Fig. 6). After the first 100 hours of immersion, the plasma-treated endocarp had a higher absorption rate and soaking. However, the time to reach in the second stage was higher compared with the untreated endocarp; that is, the plasma-treated endocarp needed more moisture to initiate the second phase of imbibition.

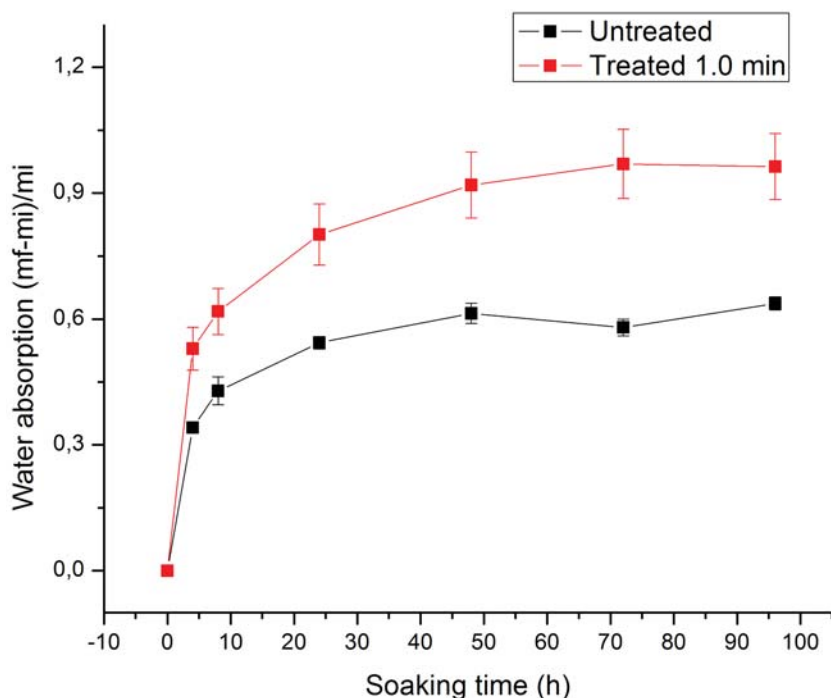


FIG. 6: Absorption of water during the immersion test for plasma-treated and untreated endocarp

Although these results indicate a greater amount of water available to the seed, it is not possible to ensure that the plasma also changed the wettability of the seed coat. For a seed to germinate, it is necessary for the medium to provide sufficient water for the activation of chemical reactions related to metabolism, thus triggering the recovery process of embryo development.¹⁸ Therefore, these results are promising for improved germination and vigor when the endocarp is treated by plasma.

The electric conductivity and pH of the exudate were monitored during the imbibition process (Fig. 7). Several studies have correlated the increase in conductivity with the decrease in germination and seed vigor.¹⁹ In addition, seeds with low viability and vigor have increased leaching of solutes that compare to vigorous seeds with high germination.²⁰

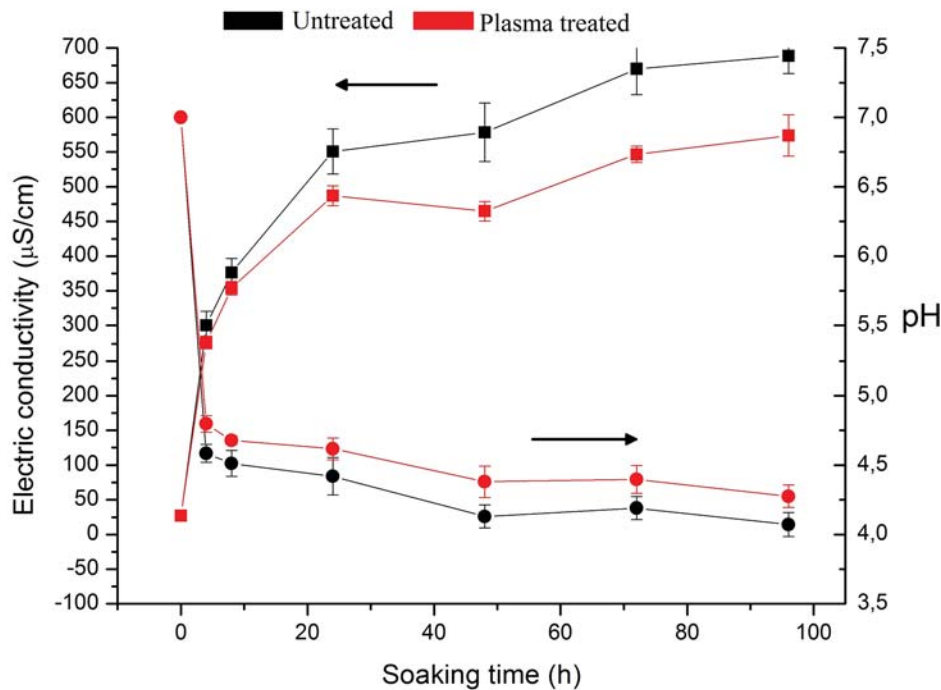


FIG. 7: Electric conductivity and pH of the water after substance exudation from the endocarp during immersion test

In our study, it was observed that the exudate of untreated endocarps showed a higher conductivity than those treated by plasma. This means that the untreated endocarp released more ions in water. The pH behavior, which depends upon the relative concentrations of H^+ and OH^- , was inversely proportional to electrical conductivity. This means that the ions released are mainly H^+ and OH^- . These ions are found dissolved in substances such as lignin, amino acids, and sugars, which are usually found in large quantities in the seeds. The pH results indicate that there was a greater release of H^+ in seeds over damaged cellular membranes, leading to the acidification of exudate. Based on these studies and the results obtained in our experiments, it is expected that plasma-treated seeds increase in germination vigor compared with untreated seeds. Spectrophotometry analysis showed an absorption band at 230 nm and 280 nm (Fig. 8), which are lignin characteristics.²¹

There is a displacement of 223 nm to 230 nm for exudation time exceeding 4 hours, probably due to lignin degradation steps. In addition, the values of absorption intensities for the two peaks increase continuously over time and the growth rate was higher in untreated endocarp. Imbibition results, electrical conductivity, pH, and leaching of substances indicate a greater vigor of the seeds contained in plasma-treated endocarp. Therefore, all present results point to a superiority of the plasma-treated endocarp with respect to the seed germination and vigor.

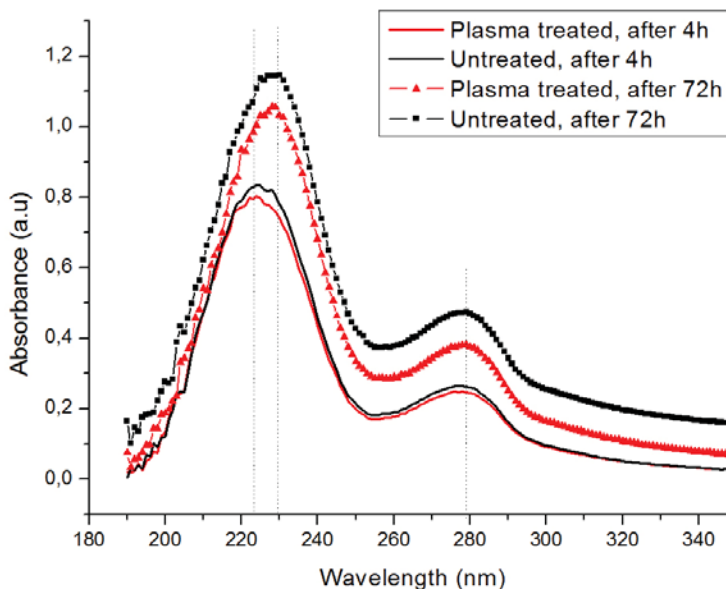


FIG. 8: Optical absorption curve obtained by UV-VIS spectrophotometry of the substance exuded from the endocarp during the immersion test

In addition to the germination tests, other parameters of physiological quality such as fresh weight, dry weight, and length of seedlings (Table 2) were determined.

The plasma-treated seeds presented a slightly lower germination rate than the untreated seeds. However, when observing the remaining quantities of physiological quality, the superiority of the plasma-treated seedlings was remarkable with regard to the percentage of humidity and length of the seedling, which were significantly higher. A possible explanation for this phenomenon is the fact that more vigorous seedlings have greater nutritional reserves available for growth. The untreated seeds had a higher exudation of substances compared with the treated seeds: due to reduced leaching of treated seeds, these showed higher reservation for seedling growth.

TABLE 2: Value of germination rate, fresh weight, dry weight, moisture, and size of the seedling

Sample	Germination (%) ¹	Fresh weight (g) ²	Dry weight (g) ³	Moisture (%) ⁴	Size (mm) ⁵
Untreated	76 ± 20	3.09 ± 0.45	0.40 ± 0.05	13	116.5 ± 51.2
Plasma-treated	71 ± 15	5.11 ± 1.13	0.53 ± 0.14	10	175 ± 39.6

¹Percentage of germinated seeds.

²Weight of full plant (foot + hypocotyl + epicotyl).

³Weight after dehydration.

⁴Water content of fresh weight.

⁵Total length of the seedling.

IV. CONCLUSION

S. tuberosa seeds, which are surrounded by rigid, woody, and lignified endocarp, were used to evaluate plasma penetration efficacy and depth of treatment in soaking and overcoming seed dormancy. Considering the obtained results, it may be concluded that:

1. Plasma was effective in increasing capillary drag of the rigid and woody endocarp with its enclosed seed.
2. Higher volumes of water were absorbed by the endocarp after plasma treatment.
3. The results of electric conductivity, pH, and leaching substances during imbibition test indicate that the plasma treatment had a positive effect on the structural stability of the endocarp and/or seed.
4. Variables of the physiological qualities, such as fresh weight, dry weight, and length of the seedlings showed that plasma treatment was effective for seed germination.

We conclude that plasma is effective even in this particular case when the seed is enclosed by the endocarp.

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