ABRATES
Journal of Seed Science
ISSN 2317-1545
www.abrates.org.br/revista

ARTICLE

Journal of Seed Science, v.44, e202244041, 2022

http://dx.doi.org/10.1590/2317-1545v44261872

Atmospheric plasma overcomes dormancy of *Pityrocarpa moniliformis* (Benth.) Luckow & R. W. Jobson seeds

Josefa Patricia Balduino Nicolau[1]*, Márcio Dias Pereira[1], Francisco Eudes da Silva[1], Dinnara Layza da Silva Souza[2], André Dantas de Medeiros[3], Charline Zaratin Alves[4]

ABSTRACT: Atmospheric plasma application technology consists of a fast and practical method, which has the potential to modify the surface of seeds with integumentary hardness. The aim of this research was to evaluate the effect of applying atmospheric cold plasma on *Pityrocarpa moniliformis* seeds, assessing its implications on the seed coat impermeability, germination and vigor. Cells were subjected to plasma for 1.5, 2.0, 3.0, 4.0 and 5.0 minutes, using seeds without any treatment as a control. After application, the seed coat wettability, imbibition curve and electrical conductivity of the soaking solution were determined. Seeds were also subjected to the germination test, and its results were used to determine viability, median, uniformity and asymmetry of germination. Seeds subjected to plasma for 5.0 and 4.0 minutes showed the lowest apparent contact angles, 64 and 61°, respectively, characterizing greater wettability of the seed coat among the treatments tested. Higher germination was observed in the treatments with plasma application when compared to the control. Atmospheric plasma application technology has the potential to be used as an accelerator of *P. moniliformis* seed germination.

Index terms: cold plasma, forest seeds, tegumentary dormancy, wettability.

RESUMO: A tecnologia de aplicação do plasma atmosférico consiste em um método rápido e prático, que apresenta potencial de modificação da superfície tegumentar de sementes com dureza tegumentar. O objetivo desta pesquisa foi avaliar o efeito da aplicação do plasma frio atmosférico em sementes de *Pityrocarpa moniliformis*, verificando suas implicações sobre a impermeabilidade do tegumento, germinação e vigor. As sementes foram submetidas ao plasma por 1,5; 2,0; 3,0; 4,0 e 5,0 minutos, utilizando-se como controle, sementes sem nenhum tratamento. Após a aplicação, determinou-se a molhabilidade do tegumento, curva de embebição e condutividade elétrica da solução de embebição. As sementes também foram submetidas ao teste de germinação, tendo-se, a partir dos seus resultados determinado a viabilidade, mediana, uniformidade e assimetria da germinação. As sementes submetidas ao plasma por 4,0 e 5,0 minutos apresentaram os menores ângulos de contato aparente, 64 e 61°, respectivamente, caracterizando maior molhabilidade do tegumento, entre os tratamentos testados. Maior germinação foi observada nos tratamentos com aplicação do plasma quando comparado com o controle. A tecnologia de aplicação do plasma atmosférico tem potencial para ser utilizada como acelerador da germinação de sementes de *P. moniliformis*.

Termos para indexação: plasma frio, sementes florestais, dormência tegumentar, molhabilidade.

***Corresponding author**
E-mail: patricia.balduino@hotmail.com

Received: 03/09/2022.
Accepted: 07/25/2022.

[1]Universidade Federal do Rio Grande do Norte, Unidade Especializada em Ciências Agrárias, Caixa Postal 07, 59280-000, Macaíba, RN, Brasil.

[2]Universidade Estadual do Piauí, Centro de Ciências Agrárias, Caixa Postal 381, 64002-150, Piauí, Brasil.

[3]Universidade Federal de Viçosa, Departamento de Agronomia, Caixa Postal 22, 36570-900, MG, Brasil.

[4]Universidade Federal de Mato Grosso do Sul, Departamento de Agronomia, Caixa Postal 549, 79.070-900, Chapadão do Sul, MS, Brasil.

INTRODUCTION

Pityrocarpa moniliformis (Benth.) Luckow & R. W. Jobson belongs to the Fabaceae family and produces seeds with physical dormancy, of the tegumentary hardness type, characterized by the resistance imposed by the structure of the seed coat, which is rich in lignin, cutin and suberin and has a superficial layer of wax. This tegumentary constitution results in hydrophobicity and restricts the absorption of water by the seed, hindering the germination process, so a treatment is required to overcome dormancy (Azerêdo et al., 2011; Maia-Silva et al., 2012; Marcos-Filho, 2015).

Physical or chemical scarifications with acids and immersion in hot water have been the most used methods to overcome the physical dormancy of *P. moniliformis* and others in the same family, which have this type of impediment to water absorption (Azerêdo et al., 2011). However, such procedures can cause injuries in the structure of seeds, reduced vigor, besides leading to increased infections by microorganisms.

As an alternative of pre-germination treatment to overcome dormancy, the technology of atmospheric plasma application in seeds has stood out. According to Alves-Júnior et al. (2017), it can act as a modifier of germination dynamics, reducing the effects of dormancy and promoting germination. Such use is justified by the fact that it is a faster, less invasive technology that does not produce residue or damage to the seeds and health of the analyst, allowing greater economic profitability.

Plasma is a gas composed of free electrons, positive and negative ions, atoms, reactive and neutral molecules and photons. It is obtained by mixing a gas, or associated gases, with an energy source, being operated in low-pressure systems or at atmospheric pressure (Loureiro and Amorim, 2016; Thirumdas et al., 2017). Its capacity to alter chemical bonds, for example, is indicative of its potential to eliminate or reduce tegumentary dormancy in some seeds, and it may promote partial breakdown of polymer chains, enabling the inclusion of new functional groups (De Groot et al., 2018; Yodpitaka et al., 2019).

Therefore, previously hydrophobic surfaces start to have a higher affinity for water, as suggested in studies that used plasma for this purpose, in seeds of *Mimosa caesalpiniafolia* (Guimarães et al., 2015) and *Erythrina velutina* Willd (Alves-Júnior et al., 2016).

The challenge for studies involving the use of plasma on living surfaces is the adjustment of the configurations and conditions of application, as well as the materials to be altered. Research indicates that factors related to plasma application, such as seed exposure time, can decisively influence the results obtained (Silva et al., 2018; Serý et al., 2020).

However, as these conditions are not yet elucidated for most species, the aim was to evaluate the effect of atmospheric cold plasma application in *P. moniliformis* seeds, assessing its implications on seed coat permeability and germination.

MATERIAL AND METHODS

P. moniliformis fruits were collected from 15 parent trees, at least 20 meters apart at the Experimental Farm of the Federal Rural University of the Semi-Arid Region (UFERSA), Mossoró, Rio Grande do Norte, Brazil (5° 03’ S and 37° 24’ W). The fruits were at early dehiscence, and, after collection, they were manually processed, by extracting and selecting healthy and well-formed seeds. These were subjected to atmospheric cold plasma application for exposure periods of 1.5, 2.0, 3.0, 4.0 and 5.0 minutes and, for comparison purposes, seeds without any treatment were used as control.

Plasma application was performed in the Plasma Laboratory of UFERSA and was generated from a dielectric barrier, produced on a coplanar plate (Figure 1). The system was powered by a high voltage source, with power of 10 kv and frequency of 400 khz (Figure 1A).

For each experimental condition, 50 seeds were used at a time in a Petri dish of 90 mm in diameter and 15 mm in height (Figure 1B). The interior of the system consisted of a phenolic paper sheet, with copper-coated electrodes (Figure 1C). After receiving plasma application, the seeds were subjected to the following evaluations.

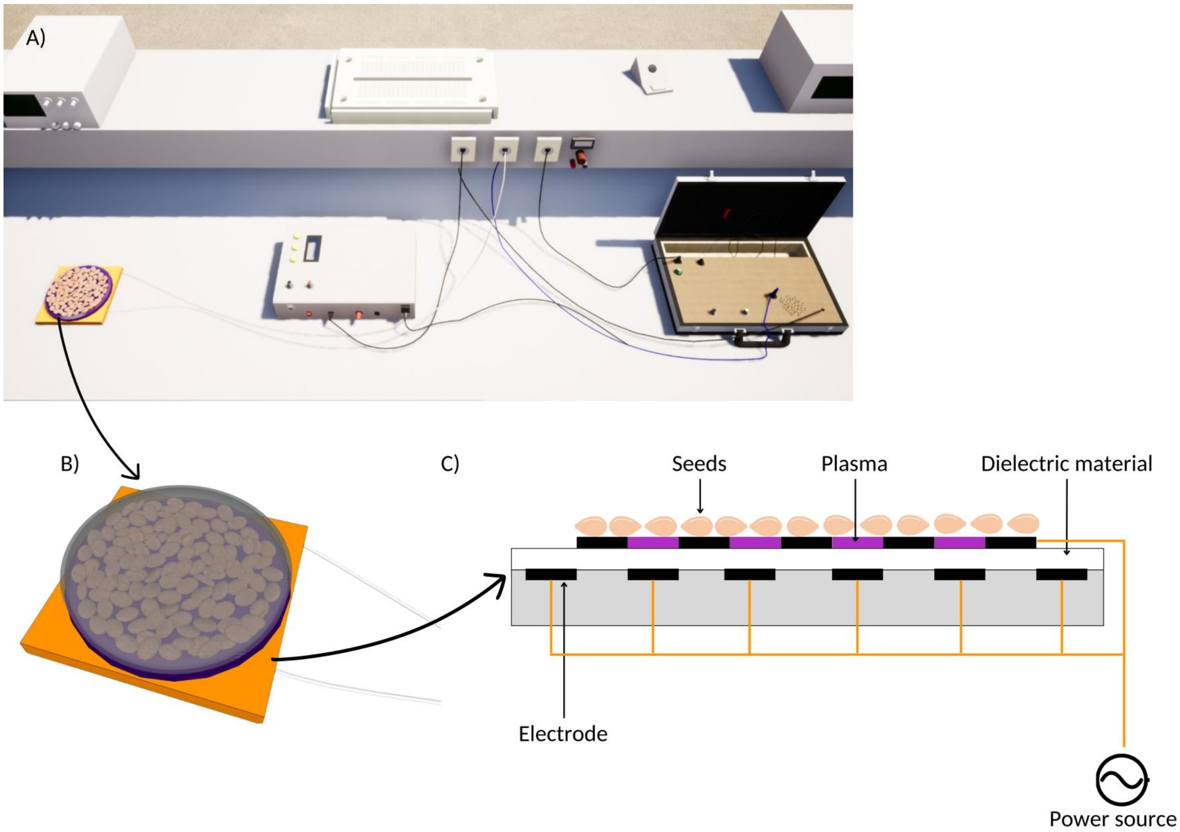

Figure 1. Schematic representation of the system used for plasma application with high voltage power source (A). Detail of *Pityrocarpa moniliformis* seeds arranged inside the glass plate under coplanar plate (B); scheme indicating the position of the electrodes and dielectric material inside the plate in relation to the seeds (C).

Determination of wettability: both seeds that were treated and those used as control had the apparent angle of contact of the seed coat with water, deposited in the form of a drop, measured by the sessile drop method, in order to indirectly evaluate the wettability of the seed coat. For this, seven seeds of each treatment were deposited on a flat surface, and a drop of distilled water was applied on the seed coat using a 100-µl micropipette. Thirty seconds after the application of the water drop, the image of the contact of the drop with the seed surface was captured, according to the methodology described by Alves-Júnior et al. (2016).

After the acquisition of the images, they were transferred to a computer and processed in Surftens® 3.5 software, Frankfurt, Germany (Cheng et al., 2014). Then, the values of the apparent contact angles, formed by the water drop and the seed coat, were obtained. These values were subjected to descriptive statistics (arithmetic mean and standard deviation). The lower the contact angle, the greater the wettable surface, so the greater the wettability of the seeds.

Water absorption curve and electrical conductivity: four replications with 50 seeds of each treatment were used, and the weight of each replication was measured on a precision analytical scale (0.001 g). Then, the seeds were put into properly identified plastic cups containing 75 mL of distilled water and placed in germination chamber, at 25 °C, with 12 h photoperiod (Pereira et al., 2015).

The relative percentage of imbibition was obtained from the variation of seed mass, after 2, 4, 6, 12, 24, 36, 48, 60, 72 and 96 h of soaking, compared to unsoaked seeds (0 h). For this purpose, a sieve was used to separate the seeds from the liquid; after that, they were placed on paper towels to remove excess water and only then their weight was measured. Along with the determination of the imbibition curve, the liquid in which the seeds were immersed was evaluated to quantify the leaching of the substances through the electrical conductivity.

The evaluations were carried out at the respective intervals, when the seeds were weighed during the imbibition period, as described above. After evaluation in each period, the seeds were put back in the same liquid in which they were immersed during the imbibition process. The electrical conductivity was determined using a Digimed DM conductivity meter and the mean values obtained for each treatment were expressed in $\mu S.cm^{-1}.g^{-1}$ of seeds.

Germination test: the substrate consisted of paper towel, moistened with distilled water in a volume corresponding to 2.5 times its dry mass. For this, 50 seeds per replication were used, totaling 200 seeds per treatment. The test was conducted in a germination chamber at temperature of 25 °C and photoperiod of 12 h (Brasil, 2013). Seeds that produced the primary root with length greater than 2 mm were considered germinated.

The cumulative germination curve was fitted according to the model proposed by Richards (1959), which characterizes the change in biological behavior from seed germination. The values obtained after calculation of Richards' function were converted by the logistic function and associated with population parameters: viability (Vi %), which indicates maximum seed germination; median (Me%), which indicates the time at which 50% of germination is achieved; dispersion (Qu), which indicates germination uniformity as a function of time in days; and (Sk), which represents the asymmetry of the Richards' curve. The formulas developed by Hara (1999) were used for the calculations.

Design and statistical analysis: the design used was completely randomized, with four replications per treatment. Analysis of variance was performed by the F test (5%) and, when significant, the means of the treatments were compared by Tukey test. The analyses were carried out in the statistical environment R (R Core Team, 2020).

RESULTS AND DISCUSSION

In seeds subjected to plasma, the exposure time had a significant effect on wettability. There was a decrease in the values of apparent contact angles, as the time of exposure to plasma increased, and seeds treated for 4.0 and 5.0 minutes had the lowest angles, 64 and 61°, respectively (Figure 2). These results make it possible to infer that plasma promoted chemical interactions in the surface components of the seed coat structure, capable of altering its relationship with water, reducing the apparent contact angle between the seed coat and the water drop.

The lowest apparent contact angle for treated seeds was that obtained when there was longer exposure to plasma (4.0 and 5.0 minutes), which implies a larger wettable surface. This result is justified by the chemical action that cold plasma obtained at atmospheric pressure exerts on the structural characteristics of biomaterials, such as seed coat.

Untreated seeds had the highest contact angle, differing from all other treatments. This indicates that plasma, in all exposure times evaluated, reduced the contact angle of the seeds with the water drops, increasing their wettability. With the increase in the time of exposure to plasma, the composition of the outer layer of the seed coat may undergo chemical modifications, at the level of dysfunctions of functional groups, capable of altering the hydrophobic profile of the seed coat and, eventually, promote increased water absorption according to Silva et al. (2018).

In general, hydrophobicity occurs in seeds that have hard coat and have waxy surface layers, formed by specific substances, which are able to block the entry of water into the seeds, inhibiting imbibition and thus slowing or preventing the germination process (Bewley et al., 2013; Serý et al., 2020).

The results found in this study are in agreement with those obtained by Alves-Júnior et al. (2016), who found that the apparent contact angle of *Erythrina velutina* seeds treated with plasma was also reduced.

The dynamics of water absorption is directly linked to wettability. Thus, there was variation in the imbibition of seeds subjected to plasma, when compared to untreated seeds, showing that this technique promoted greater wettability and, consequently, greater mass gain by *P. moniliformis* seeds (Figure 3).

During the process of water entry into the seeds, characterized in this study by means of the absorption curve, there was greater variation in the mass of seeds subjected to plasma, which absorbed a greater amount of water throughout the process. On the other hand, the control seeds had lower absorption, confirming what was described in the wettability analysis.

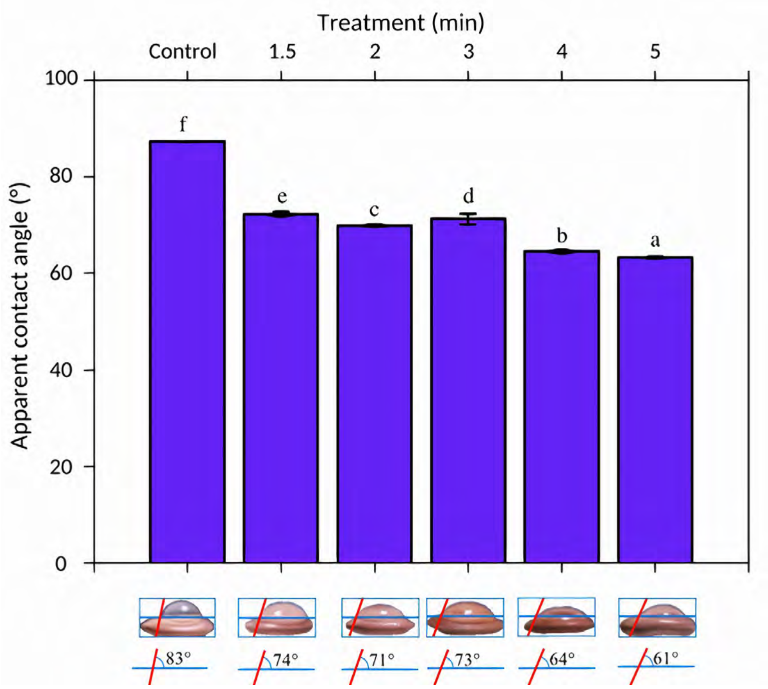

Figure 2. Apparent contact angle (°) formed by the water drop and the surface of *Pityrocarpa moniliformis* seeds subjected to different periods of exposure to atmospheric cold plasma by dielectric barrier discharge. Means followed by different letters differ from each other by Tukey test at 5% probability level.

For the species under study, this process occurred in three phases, as occurs for most seeds. Phase I was marked by the rapid imbibition period, followed by a plateau effect, with stabilization of the absorption, characterizing phase II, and phase III, whose beginning is marked by the protrusion of the primary root, as described by Bewley et al. (2013).

Therefore, seeds treated with plasma may have increased the density of hydrophilic sites on the seed coat, thus reaching phase III faster. This behavior was observed in all seeds subjected to plasma (Figure 3).

The electrical conductivity of the soaking solution of seeds treated with plasma increased under all tested conditions (Figure 4). This result may indicate greater transfer of solutes from seed cells to the soaking solution. This may have been due to the modification of the physical structure of the seed coat, allowing greater transit of water and solutes from the interior of the seeds to the solution. During the process of water absorption by the seeds, temporary structural changes occur, especially in the membranes, which results in the immediate and rapid leakage of solutes and metabolites to the solution (Bewley et al., 2013). The evaluation of the release of substances and ions by electrical conductivity determination indicates the chemical relationships in the most permeable membranes and changes in the waxy layer, which externally involves the seed coat.

The electrical conductivity of the soaking liquid of seeds subjected to plasma was higher compared to the other treatments. These results corroborate those obtained for wettability and curve of water absorption by the seeds. This increase may be related to possible changes in their chemical structure, promoted by the exposure to plasma. Thus, it allows greater contact and absorption of water and, consequently, greater leaching of the solutes, due to the release of ions, organic acids and sugars during imbibition, which are responsible for increasing the acidification of the medium. This behavior was not observed in the control seeds. These seeds, from 48 h, kept the surfaces of their coats more hydrophobic, with lower contact with water and lower and slower imbibition, which reduced the amount of leachates released to the soaking solution.

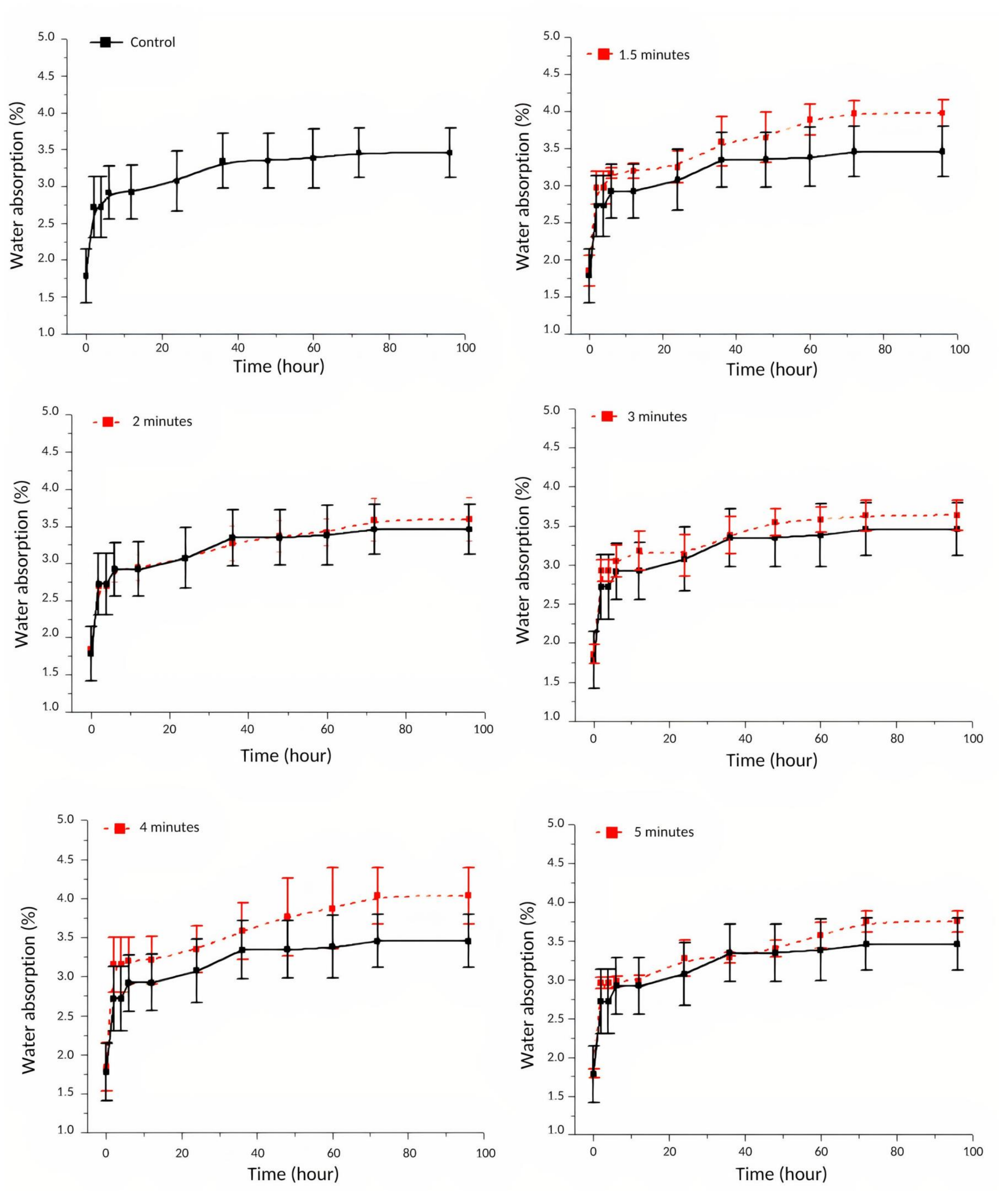

Figure 3. Mass increment in *Pityrocarpa moniliformis* seeds subjected to different periods of exposure to atmospheric cold plasma by dielectric barrier discharge during the imbibition process.

Results similar to that found here were verified by Silva et al. (2018), with *Hybanthus calceolaria* seeds treated with atmospheric plasma for different periods of exposure and imbibition. Assessing the adequacy of the conductivity test, Araújo et al. (2022) observed that the release of exudates increased as the seeds were imbibed.

Seeds exposed to plasma showed different behaviors for the cumulative germination curve (Figure 5). For untreated seeds, there was accumulation of the percentage of germinated seeds, which did not exceed 8% germination at 10 days

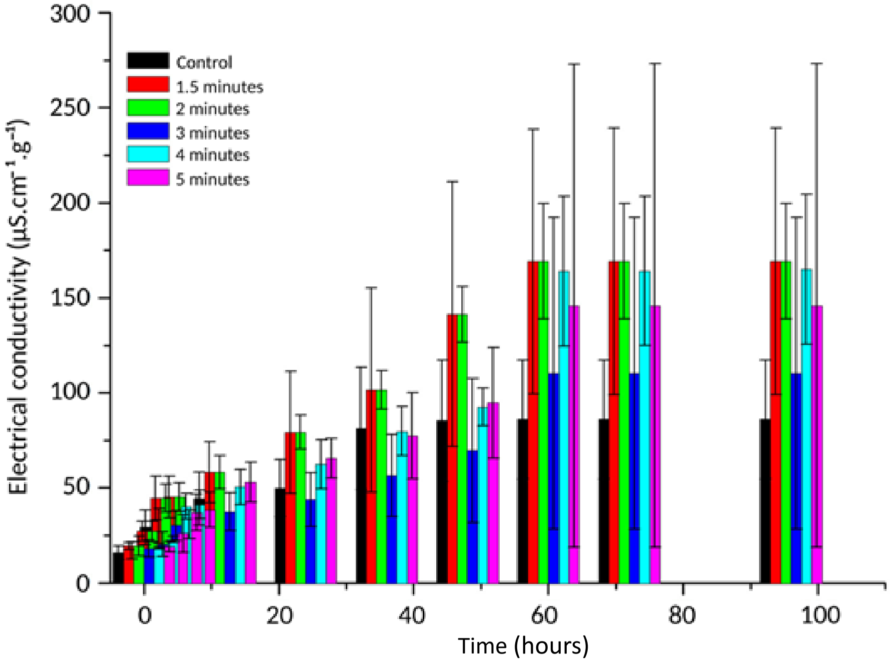

Figure 4. Electrical conductivity of *Pityrocarpa moniliformis* seeds subjected to different periods of exposure to atmospheric cold plasma by dielectric barrier discharge during the imbibition process. Bars represent 95% confidence intervals.

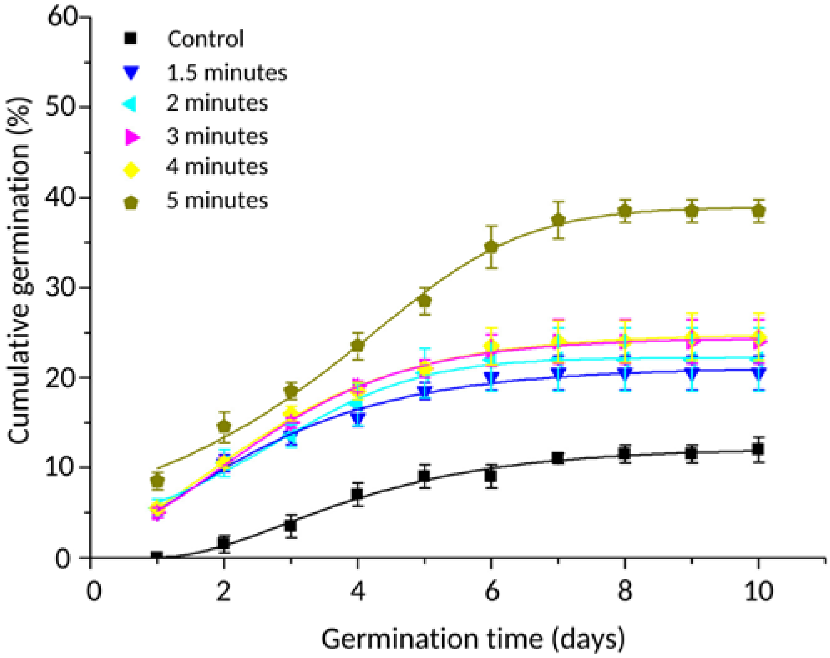

Figure 5. Cumulative germination of *Pityrocarpa moniliformis* seeds according to the time of application of atmospheric cold plasma by dielectric barrier discharge determined by method of Richards (1959).

after sowing, when germination stabilized. For seeds treated for 4.0 minutes, there was an increase in germination accumulation during the days, with percentages higher than 20% compared to untreated seeds, proving the beneficial effect of plasma on germination.

The treatment that promoted the highest cumulative germination was the exposure of seeds to plasma for 5.0 minutes. For this period, accumulation was close to 40%, representing an increase of 30% in cumulative germination, compared to the control treatment. Thus, it is clear that the exposure time influences seed germination (Figure 5).

The increase in the germination of *P. moniliformis* seeds can be justified by the action of plasma in contact with their surface because the interaction between free radicals produced by plasma and the elements that make up the seed lead to greater water absorption, which favors the germination process (Zahoranova et al., 2016; Alves-Júnior et al., 2016; Sivachandiran and Khacef, 2017). Figure 6 shows the results related to population parameters obtained through the Richards' curve for germination.

The viability values of seeds treated with plasma indicated higher germination ($p < 0.05$), compared to untreated seeds. Germination was higher for seeds treated for 5.0 minutes, whose viability was 38%, while in the control treatment, the value was 12%. Thus, it is possible to affirm that the treatment with plasma made it possible to overcome dormancy and favored seed germination.

The median (Me) estimates the time required for 50% of the seeds to germinate and characterizes the rate of this process in the studied species (Alves-Júnior et al., 2017). Seeds exposed to plasma for 5.0 minutes had the shortest average germination time among all treatments. This characteristic is very important for seedling production because the establishment of plants in the field is directly related to the initial development of seedlings. In addition, seeds treated with plasma for 5.0 minutes showed greater uniformity in germination as a function of time (QU), and this parameter is of interest for the production of seedlings of forest species, since this characteristic is an indication of seedling performance in the field.

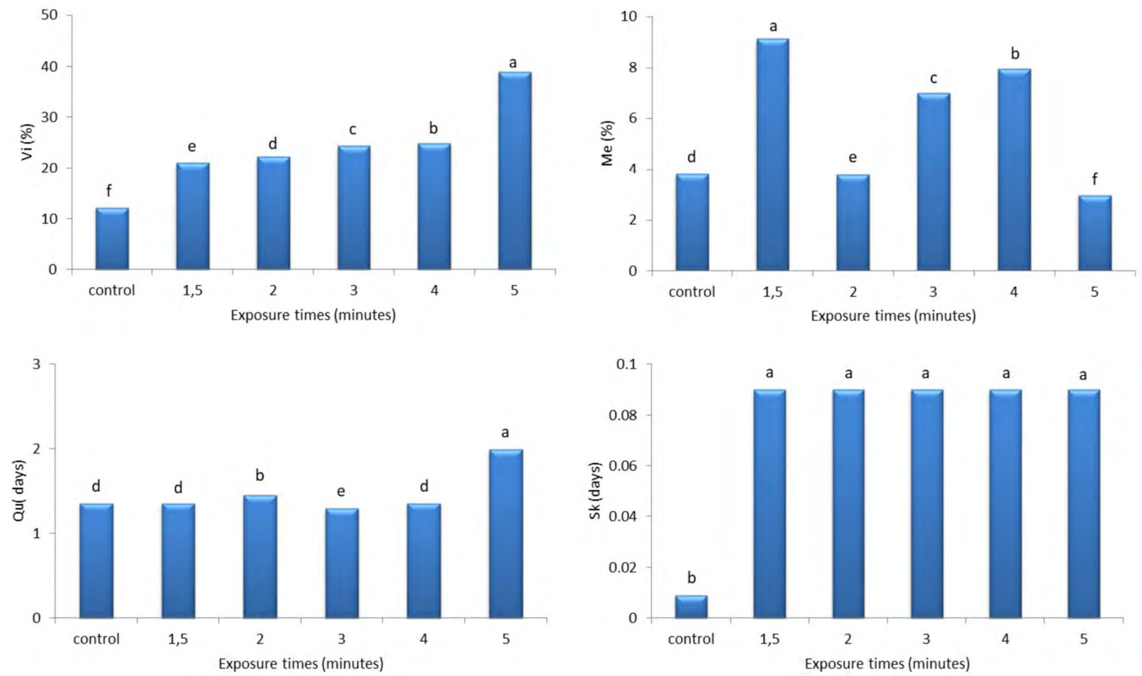

Figure 6. Population parameters of the Richards' equation: Viability - Vi (%), Median - Me (%), Uniformity - Qu (days) and Asymmetry - SK for the germination of *Pityrocarpa moniliformis* seeds subjected to plasma by dielectric barrier discharge. Means followed by different letters differ from each other by Tukey test at 5% probability level.

The values expressed by these parameters indicate changes in the biological behavior of the species after treatment with plasma, verified by the value of asymmetry (Sk), which did not occur in seeds of the control treatment. Similar results were reported by Alves-Júnior et al. (2016), which can be justified by the action of plasma on seed structure, thus promoting the entry of water.

CONCLUSIONS

Atmospheric plasma application technology has the potential to be used as a method to overcome tegumentary dormancy in *P. moniliformis* seeds.

The periods of exposure of *P. moniliformis* seeds to plasma for 4.0 and 5.0 minutes promote greater wettability and better germination performance.

ACKNOWLEDGEMENTS

This study was financed in part by the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* - Brasil (CAPES) - Finance Code 001.

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