EFFECT OF LOW-PRESSURE PLASMA TREATMENT ON THE SEED SURFACE STRUCTURE OF *Desmanthus virgatus* L. WILLD.

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ABSTRACT – Low-pressure argon plasma at a controlled temperature of 40 ºC was used to overcome seed dormancy in *Desmanthus virgatus* (L.) Willd. Treatment times were 1, 3, and 5 minutes. Infrared analysis confirmed the presence of lipids, proteins, and carbohydrates without the formation of new functional groups. The low-pressure controlled environment and the inert gas plasma changed the intensity of polar and nonpolar groups present on the seed surface. These changes directly influenced the water absorption tests because all treated seeds germinated after 24, 36, and 312 hours in the treatments of one, three, and five minutes, respectively. Germination did not occur among untreated seeds, proving the effectiveness of plasma in overcoming dormancy. The pH and conductivity results showed that plasma treatment resulted in faster germination and lower nutrient release to the medium. In addition, the seeds treated for one and three minutes showed superior results for the germination potential, germination rate, and germination index, demonstrating the effectiveness of low-pressure plasma as a clean technique and an alternative tool for reducing environmental impacts in the surface modification of *D. virgatus* L. Willd seeds.

Keywords: Argon plasma; Germination; Physical dormancy.

EFEITO DO TRATAMENTO DE PLASMA DE BAIXA PRESSÃO NA ESTRUTURA DE SUPERFÍCIE DE SEMENTES DE Desmanthus virgatus *L. WILLD.*

RESUMO – Plasma de argônio a baixa pressão a uma temperatura controlada de 40ºC foi utilizado para superar a dormência de sementes de ***Desmanthus virgatus*** *L. Willd. Os tempos de tratamento foram de 1, 3 e 5 minutos. A análise de infravermelho confirmou a presença de lipídios, proteínas e carboidratos, sem a formação de novos grupos funcionais. O ambiente controlado a baixa pressão e o plasma do gás inerte alteraram a intensidade dos grupos polares e apolares presentes na superfície da semente. Essas alterações influenciaram diretamente os testes de absorção de água, nos quais todas as sementes tratadas iniciaram o processo de germinação em 24, 36 e 312 horas, para os tratamentos de um, três e cinco minutos, respectivamente. A germinação não ocorreu entre as sementes não tratadas, comprovando a eficiência do plasma em superar a dormência. Os resultados de pH e condutividade mostraram que o tratamento com plasma resultou em germinação mais rápida e menor liberação de nutrientes para o meio. Além disso, sementes tratadas por um e três minutos apresentaram resultados superiores quanto ao potencial e taxa de germinação e índice de germinação, demonstrando a eficiência do plasma a baixa pressão como técnica limpa e uma ferramenta alternativa para a redução do impacto ambiental na modificação superficial de sementes de* ***D. virgatus*** *L. Willd.*

Palavras-Chave: Plasma de argônio; Germinação; Dormência física.

1. INTRODUCTION

Plasma technology is widely used to modify the properties of metallic, ceramic, and polymeric materials, encompassing research lines that include the production of biomaterials, the degradation of organic pollutants, and photovoltaic materials (Braz et al., 2012; Braz et al., 2019; Kan et al., 2020). This technique has also shown favorable applications in important research carried out in agriculture, such as decontamination and modification of food surfaces, deterioration of microorganisms, treatments to overcome seed dormancy, and the consequent improvement of germination (Bormashenko et al., 2015; Randeniya et al., 2015; Misra et al., 2016; Šerá and Šerý, 2018).

Desmanthus virgatus is a perennial leguminous species with high adaptability to different climatic and soil conditions and important features that favor its use in agriculture. However, its sexual propagation is compromised by seed coat dormancy, which restricts water and gas exchange in the seeds and results in slow and uneven germination, with negative effects on plant development. According to the literature, seed coat permeability is crucial as water absorption activates several biochemical processes and triggers germination. Thus, depending on seed viability, this parameter can increase germination and help overcome dormancy (Bewley, 2013; Salla et al., 2016; Simões et al., 2016; Jiang et al., 2018; Misra and Schlüter, 2019), highlighting the importance of dormancy-breaking techniques that promote water absorption.

Treatments to overcome seed dormancy include mechanical, thermal, and chemical scarification. However, despite advances in pre-germination techniques, these methods have limitations in large-scale seedling production given the possibility of reducing seed vigor, resulting in phytomass losses, damaging the embryo, and increasing the possibility of microorganism infections. Moreover, residues from chemical treatments imply an environmental burden for the ecosystem (Voegele et al., 2012; Rodrigues-Junior et al., 2014; Liu et al., 2015).

Thus, the processing of materials by low-pressure plasma allows using a controlled treatment environment that does not produce environmentally harmful waste since these environments and the plasma of a specific gas can allow specific physical and chemical processes. Plasma is formed by an electrical discharge in a low-pressure gas where different types of particles are present, such as ions, energetic electrons, neutral species, free radicals, and electromagnetic radiation (Braz et al., 2012; Alves Junior et al., 2019; Braz et al., 2019). When interacting with the seed, these components can promote physical or chemical changes that facilitate water absorption and germination. For example, the creation of microcracks on the seed coat surface breaks lignin bonds and forms polar functional groups that, along with the microcracks, facilitate the interaction with water (Dhayala et al., 2006; Šerá et al., 2010; Misra and Schlüter, 2019).

The association of these parameters with seed surface modification can increase the possibilities of existing treatments as each plasma gas provides different characteristics, such as the formation of new functional groups (nitrogen or oxygen) or the thinning or formation of cracks (argon or helium). These processes can occur through sputtering, which removes atoms from a solid due to the bombardment of ions and energetic atoms. Moreover, these changes occur on the seed surface without modifying the internal seed material, not compromising the embryonic structure as long as the adequate parameters are used for each experiment (Šerá et al., 2009; Bormashenko et al., 2013; Šerá et al., 2018).

In this scenario, infrared spectroscopy can be used to assist in modification processes, identify specific functional groups on the seed surface, and observe molecular group vibrations in the infrared region. Furthermore, it is an important tool to analyze seed samples due to the significant presence of lipids, proteins, and carbohydrates, all identified by this technique (Kan et al., 2020). Therefore, understanding which types of surface changes occur on the seed structure by the action of plasma can help understand the mechanisms of water absorption by seeds, which affect germination.

From this perspective, this study aimed to propose a protocol for the use of low-pressure plasma technology with argon gas at the controlled temperature of 40 ºC as a sustainable technique to improve pre-germination treatments in seeds of *D. virgatus*.

2. MATERIAL AND METHODS

2.1. Experimental apparatus

Seed modification by plasma occurred using a conventional plasma nitriding reactor with a direct-current power source, a maximum voltage of 1500 V, and a maximum current of 2 A. The reactor had a cylindrical vacuum chamber made of AISI 304 stainless steel and measured 30 cm in diameter and 40 cm in length.

The seeds were placed in the holes of a disk made of AISI 304 stainless steel with a diameter of 75 mm and a thickness of 6 mm. Five seeds were placed in each of the 19 holes measuring 9 mm in diameter, totaling 100 seeds per treatment. An argon atmosphere was used at a flow rate of 10 sccm and a temperature of 40 ºC.

Treatments consisted of the following experimental conditions: control (seeds without plasma treatment) and seeds treated with low-pressure plasma and argon atmosphere at 10 sccm for one minute, three minutes, and five minutes at the controlled temperature of 40 ºC.

2.2. Water absorption, pH, and electrical conductivity

Four replications of 25 seeds per treatment were placed in 250-mL plastic cups containing 200 mL of distilled water.

Then, the cups were put in a germination chamber with a daily 12-hour light / 12-hour dark cycle at a constant temperature of 25 °C, according to the methodology described by Queiroz (2012).

In the imbibition test used to infer the relative water absorption, seed mass was determined using an analytical balance at three, seven, and 12-hour intervals on the first day, followed by 12-hour intervals until the third day, and ending with measurements every 24 hours until the end of the experiment, after twenty-three days.

Before each measurement, the seeds were placed on sterilized paper sheets to remove the excess water. Then, the relative water absorption rate (imbibition) was defined according to the following equation (1).

$$E(\%) = \frac{[(m_t - m_o)]}{m_o} x100\% \qquad \text{Eq.1}$$

Where: mo is the initial seed mass and mt is the total seed mass for each measurement interval.

After weighing the seeds, the imbibition liquid containing leached substances was used to measure the electrical conductivity (CE) and the pH at the same intervals mentioned before. CE measurements were performed with an Oakton PC 450 conductivity meter, and the measuring unit was given in μScm^{-1}. The pH measurements occurred immediately after conductivity analysis using a pH meter.

2.3. Germination test

The germination test was conducted with 100 seeds divided into four replications of 25 seeds, according to the methodology described by Queiroz (2012). Gerbox® boxes containing two sheets of Germitest® paper were used for seed germination, and the sheets were moistened with distilled water until 2.5 times their dry weight. Then, the boxes were put in B.O.D (Biochemical Oxygen Demand) growth chambers at a constant temperature of 25 °C with a daily 12-hour light / 12-hour dark cycle for twenty-three days.

The seedlings with the potential to resume their development and originate healthy plants under favorable conditions were considered normal in the germination counts (Mapa, 2009). The germination rate was counted daily and at the same time from the first to the twenty-third day after sowing using the following equation (2):

$$G\ (\%) = \frac{N_s}{N_o} x100\ \% \qquad \text{Eq. 2}$$

Where: Ns is the number of germinated seeds and No is the number of seeds sown.

The germination potential was determined according to the following equation, which considers the number of seeds germinated on the first day of counting, as suggested by Ling et al. (2014).

$$PG(\%) = \left({}^{N_s}/_{N_o} \right) * 100 \qquad \text{Eq.3}$$

The germination speed index refers to the time for seed germination and is calculated according to the equation suggested by Ling et al. (2014).

$$G_i = \sum (N_s | days) \qquad \text{Eq.4}$$

The experiment was arranged in a completely randomized design, and the results were compared by the Tukey test at 5% probability to analyze the means within each experimental condition. The statistical analyses were performed using the software Origin 8.0.

2.4. Infrared Spectroscopy

The infrared experiments were performed using a VERTEX 70V spectrometer from Bruker Optics equipped with a Platinum ATR (Total Attenuated Reflectance) accessory. The spectra were collected from 600 to 3,600 cm^{-1} with 180 scans and a spectral resolution of 2 cm^{-1}.

3. RESULTS

3.1. Infrared Spectroscopy

Figure 1 shows the ATR-FTIR spectra of untreated and treated seeds. There were no changes in the chemical structure of the treated seeds in relation to the presence of new functional groups. However, there were differences between the intensities of these groups, highlighting the action of plasma while maintaining the chemical structure of the seed (Figure 1).

Source: Prepared by the authors.
Fonte: Elaborado pelos autores.

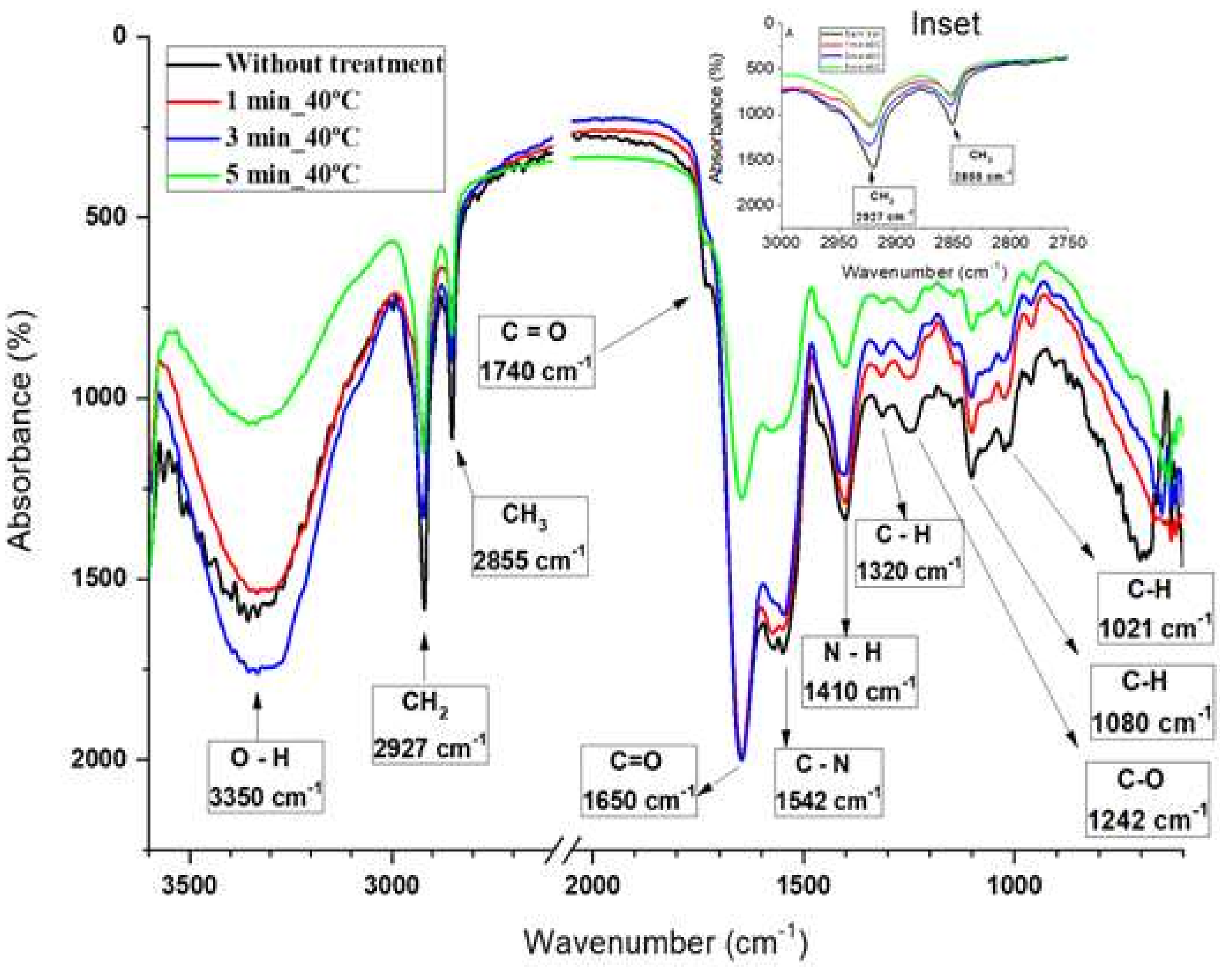

Figure 1 – ATR-FTIR spectra of untreated and treated seeds in the spectral region from 600 to 3600 cm^{-1} with the magnification of the inset spectral region from 2750 cm^{-1} to 3000 cm^{-1}.
Figura 1 *– Espectros de ATR-FTIR de sementes sem tratamento e tratadas com região espectral de 600 a 3600 cm^{-1}. Com a ampliação no inset a região espectral de 2750 cm^{-1} para 3000 cm^{-1}.*

The band at 3350 cm^{-1} refers to the O-H stretch, predominantly of liquid water. Figure 1 shows that the seeds treated for three minutes had a higher absorption peak for this stretch mode. The bands at 2927 cm^{-1} and 2855 cm^{-1} refer to the C-H stretch (CH_2 and CH_3, respectively). Figure 1 shows a gradual decrease over time in the intensity of these bands (2927 cm^{-1} and 2855 cm^{-1}) for all treated seeds.

In the spectral region from 500 to 1800 cm^{-1}, the bands of the amide groups are observed at 1740 cm^{-1}, 1650 cm^{-1}, 1542 cm^{-1}, and 1410 cm^{-1}, referring to stretches C=O, C=O, and C-N, and the N-H bending, respectively. Interestingly, the graph for the bands at 1650 cm^{-1} (stretches C=O, C-N, and N-H) shows that the intensity only decreases for the seeds treated for five minutes, while the untreated seeds or those treated for one and three minutes remain at the same level. This is the only band with this behavior in the graph.

With regard to the bands at 1542 cm^{-1} (C-N) and the N-H bending, there is a slight decrease in intensity for the seeds treated for one and three minutes and a significant decrease for those treated for five minutes. For the band at 1320 cm^{-1}, the decrease refers to the C-H deformation of hemicellulose, whereas, at 1242 cm^{-1}, it refers to the C-O stretch and the C-H bending from amide III (Liu et al., 2015). The bands at 1080 cm^{-1} and 1021 cm^{-1} may refer to the C-H (pyranose structure) and the C-H bending from aromatic structures (Abugoch et al., 2011).

3.2. Water absorption test

Figure 2 shows the water absorption curve during germination as a function of time. This process can be understood in different stages, in which the seed changes its physical structure to absorb water and triggers physiological mechanisms inherent to the beginning of germination.

In the graph, primary root emergence or the beginning of germination is indicated by the cessation of water absorption. There was no mass increase in the untreated sample, following without primary root emergence until after 576 hours.

Source: Prepared by the authors.
Fonte: Elaborado pelos autores.

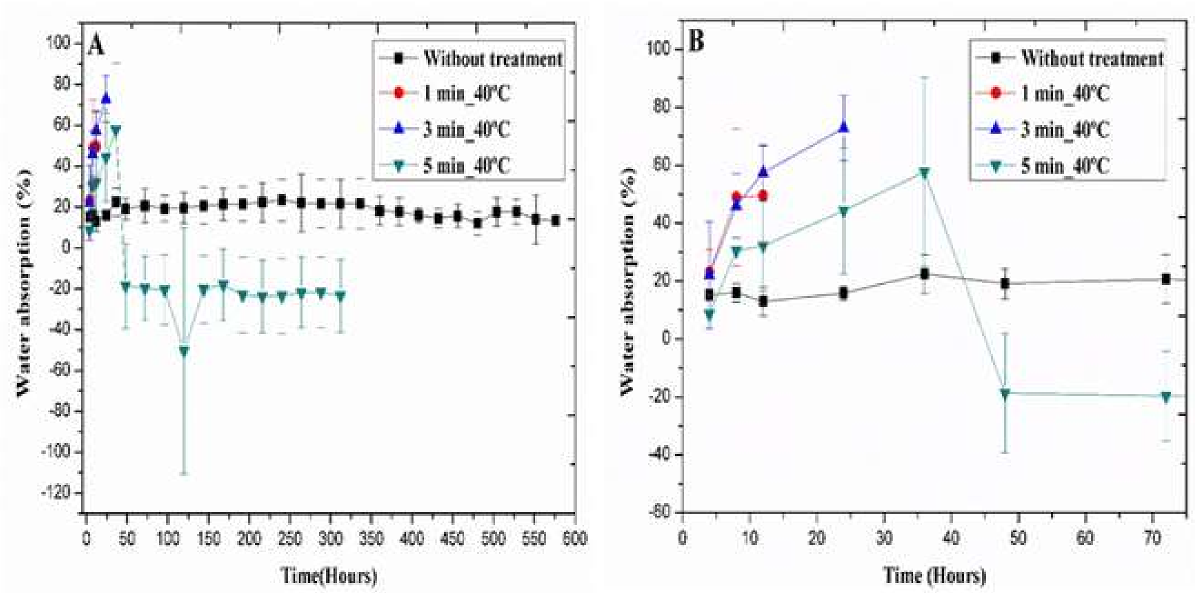

Figure 2 – Water absorption curve over time for seeds of *D. virgatus*. Complete soaking test (A). Decrease of the hour axis for better visualization of the primary root emergence point (B).
Figura 2 – *Curva de absorção de água ao longo do tempo para sementes de* ***D. virgatus****. Teste de imersão completo (A). Diminuição do eixo das horas para melhor visualização do ponto de emissão da radícula (B).*

The seeds treated for one minute showed a mass increase of about 48%, and their primary root emerged after 12 hours. The mass of the seeds treated for three minutes increased by 72%, and primary root emergence occurred in 24 hours. Finally, the seeds treated for five minutes showed a different behavior, with an initial 57% increase in absorption for 36 hours followed by an 18% decrease for the next 48 hours, remaining negative until the primary root emerged after 312 hours.

3.3. pH and electrical conductivity

Figure 3 shows the pH and electrical conductivity results of the solution analyzed during the water absorption test. In Figure 3-A, since there was no primary root emergence among untreated seeds, the measurements were performed until the end of the test, after 576 hours. There were minimal changes in conductivity during the first 48 hours, increasing linearly throughout the test. This parameter began with 7.728 ± 0.477 and ended with 55.487 ± 21.183 mS.cm^{-1}.

Similar to conductivity, the pH also showed small values in the initial 24 hours. However, there were several subsequent fluctuations after 36 hours, beginning at 5.853 ± 0.029 and ending at 6.823 ± 0.304, ranging from slightly acidic to neutral.

Source: Prepared by the authors.
Fonte: Elaborado pelos autores.

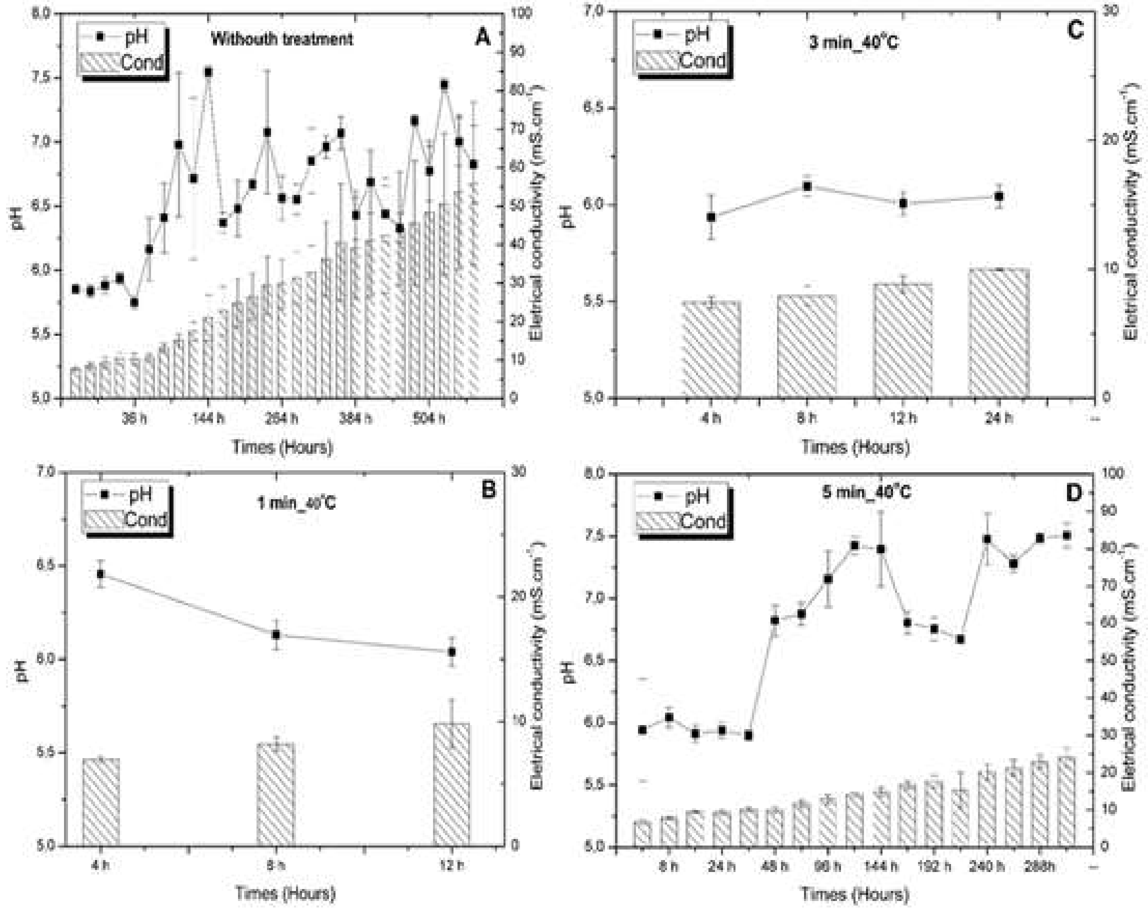

Figure 3 – Behavior of pH and conductivity during the water absorption test of *D. virgatus*. Untreated condition (A); Seeds treated with plasma for one minute at 40 ºC (B); Seeds treated with plasma for three minutes at 40 ºC (C); Seeds treated with plasma for five minutes at 40 ºC (D).

Figura 3 *– Comportamento dos parâmetros de pH e condutividade no teste de absorção de água de* ***D. virgatus****. Condição sem tratamento (A); Sementes tratadas em plasma por 1 minuto a 40 ºC (B); Sementes tratadas em plasma por 3 minutos a 40 ºC (C) e Sementes tratadas em plasma por 5 minutos a 40 ºC (D).*

The primary root of the seeds treated for one minute emerged after 12 hours, and this sample showed no significant changes in the pH and conductivity, with a slightly acidic pH and low conductivity compared to untreated seeds (Figure 3-A and 3-B). The pH was close to 6.0, and the conductivity was close to 9.8 $mS.cm^{-1}$, the latter being well below the final value of untreated seeds, 55.487 ± 21.183 $mS.cm^{-1}$.

The seeds treated for three minutes showed primary root emergence after 24 hours. Similar to the seeds treated for one minute, this sample showed no abrupt pH or electrical conductivity changes. The seeds treated for five minutes showed divergent values in relation to the previously mentioned treatments, lasting 312 hours. The pH began at 5.943 ± 0.41 and ended at 7.507 ± 0.097, remaining slightly neutral. Conductivity increased linearly over time, beginning with 6.703 ± 0.65 and ending with 24.08 ± 2.606, similar to the untreated seeds.

3.4. Germination

Table 1 shows the results of the germination parameters of seeds of *D. virgatus*.

Although each group showed a specific characteristic, all treated seeds germinated, with similar water absorption, pH, and conductivity results, directly affecting germination. The seeds treated for three minutes showed the best germination results, with the highest germination rate, germination potential, and germination index values. The lowest values were achieved by the samples treated for one and five minutes.

4. DISCUSSION

The non-occurrence of new functional groups and the change in band intensity were theoretically expected since plasma treatment occurred in a low-pressure reactor with inert argon gas, resulting in a controlled environment. Low-pressure environments remove the water adsorbed on the seed surface, facilitating the formation of microcracks, increasing roughness, and providing a larger surface area, resulting in a more significant interaction with air humidity after plasma treatment. The surface modification caused by this treatment resulted in satisfactory water absorption and, therefore, higher germination rates than other treatments, not negatively affecting the restructuring of cell membranes during imbibition since the germination potential was high. On the other hand, the results obtained with the five-minute treatment were lower as this treatment compromised the germination potential due to membrane disruption during soaking, also affecting the electrical conductivity and pH of the soaking solution (Misra et al., 2016; Barbedo et al., 2018; Nonogaki, 2018; Inocente and Barbedo, 2021).

These surface changes and interactions with water can be observed through the behavior of the band present at 3350 cm^{-1}, referring to the O-H (3350 cm^{-1}) stretch. This behavior suggests that the seeds treated for three minutes may have had a larger surface area due to sputtering, resulting in higher absorption of air moisture. In plant tissues, this can also come from the O-H groups of carbohydrates, which are part of the chemical composition of seeds and other plant parts (Pietrzak and Miller, 2005; Czekus et al., 2019; Kan et al., 2020).

The different binding energies of the functional groups observed constitute an important analysis tool since there was a gradual decrease in the intensity of the 2927 cm^{-1} and 2855 cm^{-1} bands referring to the C-H stretch (CH_2 and CH_3, respectively), mainly from lipids. This decrease is due to the longer treatment time, the higher number of collisions on the seed surface, and, consequently, the greater breakdown of C-H bonds, resulting in a linear decrease in intensity

Table 1 – Plasma effects on the germination parameters of *D. virgatus*.
Tabela 1** – Efeitos do plasma nos parâmetros de germinação de **D. virgatus.

	Germination parameters		
Condition	**Germination potential**	**Germination Percentage**	**Germination index**
Without plasma	0^{d}	0^{d}	0^{d}
Argon plasma 40º - 1 min	25^{c}	34.67^{b}	33^{c}
Argon plasma 40º - 3 min	30^{b}	42.67^{a}	40^{b}
Argon plasma 40º - 5 min	15^{a}	20^{c}	20^{a}

* Different lowercase letters in the columns indicate a statistical difference by the Tukey test (P<0.05).
**Diferenças entre letras minúsculas nas colunas indicam diferença estatística pelo teste de Tukey(P<0.05).*

for these functional groups. The other groups shown in Figure 1 (O-H, C=O, and C-N) showed a different behavior, highlighting the binding energy influence of each functional group (Pietrzak and Miller, 2005; Šerý et al., 2020).

According to García-Salcedo et al. (2018), the bands referring to stretches C=O (1740 cm^{-1}), C=O (1650 cm^{-1}), C-N (1542 cm^{-1}), and the N-H bending (1410 cm^{-1}) are particularly important as they represent the functional groups present in the sample and are used to quantify proteins and reveal changes in the structure of secondary proteins.

The intensity of the C=O (1650 cm^{-1}) and C-N bands and the N-H bending slightly decreased for the seeds treated for one and three minutes, with a significant decrease for those treated for five minutes. These polar functional groups have a hydrophilic behavior and are important for the interaction of the material with water (Santana et al., 2019). Most functional groups shown in Figure 1 are nonpolar compounds, such as C-H. Therefore, the decrease in their intensity and the permanence of the mentioned polar groups can cause more significant intermolecular interactions with water. Thus, the seeds treated for one and three minutes gained prominence in this regard, which is proven by the higher intensity obtained by the band present at 3350 cm^{-1} (stretch O-H), referring to water adsorption from air humidity.

The C=O bands (1650 cm^{-1}) showed higher average binding energy values than the C-N bands (1542 cm^{-1}) due to the double bond and the electronegativity of oxygen. C-H bonds have lower binding energy than C=O (1650 cm^{-1}). Accordingly, C-H functional groups suffered the most changes in their intensities due to their wider distribution and long carbon chains, increasing the probability of shocks compared to other functional groups. Therefore, the longer sputtering time of the five-minute treatment confirmed the broken bonds caused by the argon gas plasma.

There were also visible bands for the C-H deformation of hemicellulose (1320 cm^{-1}), the C-O stretch (1242 cm^{-1}), the C-H pyranose structure (1080 cm^{-1}), and the C-H aromatic structures (1021 cm^{-1}) (Abugoch et al., 2011; Liu et al., 2015). The intensity of these bands decreased gradually with the increase in treatment time, and the lowest values were obtained by the seeds treated for five minutes. As mentioned before, the longer treatment time may have caused more intense wear on the seed surface, inhibiting the intensity of these functional groups. Therefore, the degradation of the functional groups present on the seed coat affects the germination potential due to changes in the rate of water entry, disrupting the process and affecting important water transport structures (Barbedo et al., 2018; Nonogaki, 2018; Inocente and Barbedo, 2021).

The constant absorption values of untreated seeds occurred due to the low interaction of water with the seed surface. The untreated seed spectrum showed no decrease in the intensity of the C-H functional groups compared to the treated samples. However, all treated seeds showed gains in water absorption and primary root emergence, proving that changes in the intensity of the functional groups along with the formation of cracks caused by plasma promoted a greater interaction between the seed surface and water molecules. Moreover, the intensity of the OH band (3350 cm^{-1}) in the three-minute treatment relates to a greater interaction with water followed by primary root emergence, with lower dispersion values than the seeds treated for five minutes. Therefore, the three-minute treatment showed greater uniformity.

The different behavior of the seeds treated for five minutes can be explained by the decrease in their intensity compared to other spectra. In addition, the longer treatment time may have caused greater surface degradation, resulting in water release and nutrient loss from the seeds. As a result, important plant structures were affected and germination was compromised.

The linear increase in conductivity highlights the release of charged compounds from the seeds (ions and free radicals). With regard to the pH, the fluctuations observed during the test may be related to the attempt by the seeds to maintain the acid-base balance through biochemical processes, releasing and retaining compounds that change the pH. This behavior is characteristic of biological systems, in which the cell releases conjugate acid-base pairs (Salis and Monduzzi, 2016).

In the initial hours of the test, both conductivity and pH showed stable values, with similar behaviors for the seeds treated for one and three minutes, followed by the subsequent emergence of the primary root. This indicates that the physicochemical changes caused by plasma provided a more suitable environment that accelerated root emergence, with reduced nutrient release to the environment and better conditions for germination (Tajbakhsh, 2000). On the other hand, the seeds treated for five minutes showed a longer time for primary root emergence, greater pH fluctuation, and significant nutrient losses.

The germination process confirms these results as the seeds treated for one and three minutes showed higher germination values, the highest of which are attributed to the three-minute treatment. The two treatments differ first in relation to the time for primary root emergence and second in relation to water absorption. The primary root of the seeds treated for one minute emerged before, with less water absorption and primary root emergence after 24 hours. The seeds treated for three minutes showed a linear water absorption behavior and primary root emergence after 36 hours. The higher water absorption may have interfered with the optimization of the biochemical processes and resulted in more significant germination results for this treatment (Šerá et al., 2008; Šerá et al., 2010; Li et al., 2016).

It should be noted that the untreated seeds did not germinate, proving the effectiveness of plasma treatment to increase germination, as observed in other studies (Dhayala et al., 2006; Selcuk et al., 2008; Šerá et al., 2010; Bormashenko et al., 2015; Yodpitak et al., 2019).

The seeds treated for five minutes showed lower germination results compared to other treatments, with continuous water absorption for 36 hours followed by nutrient release to the medium for the next 312 hours. These results are subsidized by pH and conductivity changes: the first showed abrupt variations, while the latter showed a linear increase, suggesting a less favorable environment for germination (AOSA, 1983; Tajbakhsh, 2000). The pH and conductivity data show that the release of compounds occurred for a longer time in the untreated seeds, which may be related to membrane deterioration and the consequent non-elongation and protrusion of the primary root (Silva et al., 2018).

5. CONCLUSIONS

Low-pressure plasma technology using argon gas at the controlled temperature of 40 ºC effectively improved seed germination in *D. virgatus*. The treatments overcame dormancy since all treated seeds germinated. In contrast, the untreated seeds did not germinate.

AUTHOR CONTRIBUTIONS

Danilo Braz: conceived the ideas, designed the methodology, data analyze, text written and translation.

Dinnara Silva and Rômulo Sousa : conceived the ideas, designed the methodology and contributed to the writing.Mérik Rocha-Silva: technical review.

Renan Moncão: contributed to designed the methodology.Cleânio Lima and Maria Verônica Andrade: designed the methodology, Material preparation and contributed to the writing. All authors read and approved the final manuscript.

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