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Cold plasma is effective at overcoming dormancy and maintaining germination in *Pityrocarpa moniliformis* after storage[1]

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ABSTRACT - The use of cold plasma is effective in overcoming seed dormancy. However, its effects, both in the long-term and during storage, is still not fully understood. The aim of this study, therefore, was to evaluate the immediate and post-storage effect of atmospheric plasma on the seeds of *Pityrocarpa moniliformis* in overcoming seed coat dormancy. The seeds were treated with plasma for 0, 1.0, 2.0, 3.0, 4.0 and 5.0 minutes. Following application, 50% of the seeds were stored for two years while the remainder were submitted to immediate analysis. The seeds were evaluated for germination and the germination speed index, in addition to seedling length and dry weight. The experimental design was completely randomised, and included regression analysis. The plasma treatment resulted in an increase in the germination rate and vigour of the seeds, both immediately after exposure and after two years of storage. In particular, the seeds treated for five minutes showed around 39% germination, which was outstanding compared to seeds from other exposure times. Plasma is effective in overcoming seed coat dormancy in seeds of *P. moniliformis* treated for five minutes and tested immediately. The effect of plasma on the seeds of *P. moniliformis* after storage was less for all the conditions under test.

Key words: Atmospheric plasma. Germination potential. Forest species. Post-storage viability. Tegumentary dormancy.

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INTRODUCTION

Plasma is formed by an electrical discharge in a low-pressure gas containing various particles such as ions, energetic electrons, neutral species, free radicals and electromagnetic radiation (Alves Junior *et al*., 2016). This ionised gas is important in several areas due to its ability to promote changes in the properties of many materials. In the seed sector, it is mainly used to overcome seed coat dormancy, increasing water absorption capacity and the percentage of germinated seeds (Nicolau *et al*., 2022; Silva *et al*, 2017).

The positive effect of using atmospheric plasma has been demonstrated in forest seeds of *Mimosa caesalpiniaefolia* (Silva *et al.*, 2017), *Pityrocarpa moniliformis* (Nicolau *et al*., 2022) and *Desmanthus virgatus* (Braz *et al*., 2022). When in contact with the seed, the action of the plasma can promote physical or chemical changes that facilitate water absorption and, consequently, the germination process. This is due to micro-cracks that are created on the surface of the seed coat or its protective layer, and which vary with the duration and intensity of the application.

Exposing seeds to plasma can increase their survival; moreover, due to its composition, the plasma causes no toxicity or residue as its components can be found in nature (Serý *et al*., 2020). It is important to note that the plasma can penetrate to a depth of 10 nm, which limits its effect to the surface of the seed (Guo *et al*., 2018).

The long-term effects of plasma, such as its ability to maintain the changes it induces for longer, are not yet understood, as a result, the effect of plasma on stored seeds is not known. Proper storage allows the seeds to remain viable over time (Bareke *et al*., 2022); however, it is necessary to consider the inherent characteristics of each species.

The seeds of *P. moniliformis* exhibit normal physiological behaviour that favours their viability over long periods. It is recommended that the seeds be stored for up to 210 days in glass or plastic packaging in a controlled environment at a temperature of 18 ºC – 20 ºC and relative humidity of ±60% (Benedito *et al*., 2011). The species is of socioeconomic importance, fast-growing, has multiple uses, and is suitable for the restoration of degraded areas. The species is however dormant due to the seed coat being impermeable to water (Azerêdo, Paula; Valeri, 2011) (Azerêdo, Paula; Valeri, 2011).

In view of the above, the aim of this study was to evaluate the immediate and post-storage effect of atmospheric plasma on the seeds of *P. moniliformis* in overcoming seed coat dormancy.

MATERIAL AND METHODS

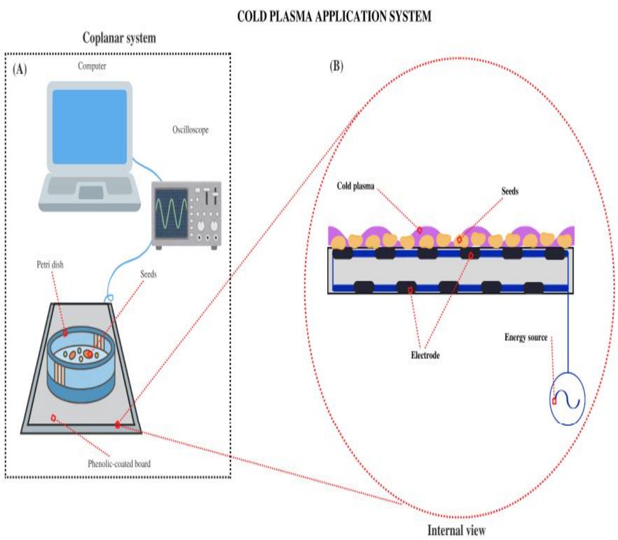
Ripe fruit of *P. moniliformis* were collected from a population of 10 individuals, approximately 15 m apart, at the Experimental Farm of the Federal Rural University of the Semi-arid Region (5º03' S and 37º24' W), in Mossoró, Rio Grande do Norte, in the northeast of Brazil.

The seeds were treated with plasma for different periods in the Plasma Laboratory of UFERSA. The system consisted of a dielectric barrier manufactured on a coplanar plate with a phenolite interior and copper-coated electrodes connected to a high-voltage source with a power of 10 kv and a frequency of 400 kHz. (Figure 1).

The treatments consisted in exposing the seeds to plasma for different periods: 0, 1.5, 2.0, 3.0, 4.0 and 5.0 min. Two hundred seeds were used for each exposure time. These were divided into four equal parts and distributed with no overlapping over the surface of the plate in a coplanar system. The seeds were then covered with a petri dish ensuring the same conditions for each treatment.

After the seeds had been exposed to the plasma for the different periods, they were divided into two equal parts. One part was analysed immediately after treatment, while the other was stored in kraft paper bags, duly identified by treatment. These seeds were then stored for two years in a controlled environment at a temperature of 20 ± 2 ºC and relative humidity of 80%. After the two-year storage period, the seeds underwent the same analyses and evaluations as the other treated seeds.

Figure 1 - Seeds of *P. moniliformis* submitted to an application of dielectric barrier plasma in a coplanar system at a frequency of 10 kv and covered by a Petri dish (A), and internal representation of the dish showing the arrangement of the seeds (B)



The germination test comprised four replications of 50 seeds, using three sheets of paper towel as substrate, moistened with distilled water at 2.5 times the dry weight of the paper. The seeds were arranged in rolls and incubated for 21 days in a germination chamber set to 25 °C and a 12-h photoperiod (Brasil, 2013).

The following variables were analysed: (a) germination - rate of normal seedlings, (b) germination speed index (GSI) - calculated as per Maguire (1962) by means of daily counts of the number of seeds that emitted a primary root (> 2 mm), and length (cm) of the shoots and root system - measured in mm using the ImageJ® software.

The experimental design was completely randomised, with four replications per treatment. Regression analysis was used for the quantitative data. The analyses were carried out using the R statistical software (R Development Core Team, 2020).

RESULTS AND DISCUSSION

The plasma promoted an increase in the germination rate and vigour of the seeds of *P. moniliformis*. This behaviour was seen both in the seeds that were tested immediately following exposure to the plasma and in those stored for two years (Figure 2). The seeds that were treated and were evaluated immediately after application, particularly those subjected to the five-minute exposure, showed a high germination rate (39%), compared to the other exposure times.

At the end of the storage period, the values for germination and vigour were lower than for the seeds that were treated and immediately evaluated. The seeds that were exposed to the plasma for five minutes and then stored achieved 20% germination compared to the control group, where germination was 12%.

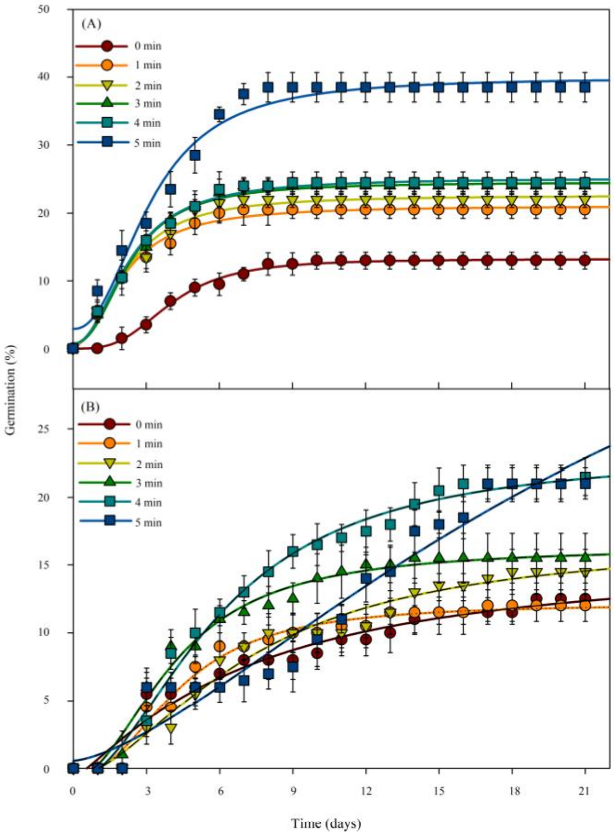
The use of plasma in seeds is affected by such factors as the source of the plasma, the plant species, exposure time, feed gas and water content of the seeds (Serý *et al.*, 2012). Based on the same settings and exposure times, Nicolau *et al.* (2022) reported results that corroborate this study, using seeds from the same species subjected to similar treatment in terms of the application system, frequency and period.

The positive effect of plasma on germination may be due to the action promoted by the treatment, significantly altering some structures in the seed (Waskow; Howling; Furno, 2021). Plasma can accelerate the germination process (Jiang; Li; Dong, 2018), since, when in contact with the surface of the seeds, it causes changes in the structure of the seed coat, affording greater water absorption, and, consequently, favouring the germination process (Adhikari *et al.*, 2020). In addition, it can promote growth of the shoots and roots (Jiang; Li; Dong, 2018), resulting in more resistant plants (Măgureanu *et al.*, 2018) with branched roots (Mildaziene *et al.*, 2018).

With regard to the uniformity of the germination speed index, the pattern was linear, both for the seeds treated and evaluated immediately and for those undergoing storage. However, for the seeds treated and evaluated immediately, the uniformity of germination increased with the exposure time, showing values of 13.34 and 1.10 when exposed for five and zero (control) minutes, respectively. A similar pattern was seen for the treated stored seeds, where the process occurred more slowly, those treated for five minutes showing greater uniformity (6.7) compared to the control (0.05), as shown in Figure 3.

Considering that the seeds of *P. moniliformis* show normal physiological behaviour, maintaining their viability over long periods (Carvalho, 2010), the increase in uniformity can be attributed, regardless of storage, to the beneficial effects of the plasma treatment.

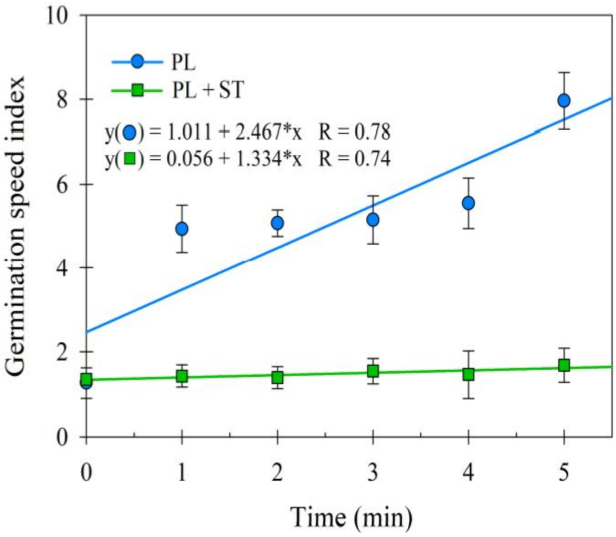
Figure 2 - Initial effects of plasma on germination in seeds of *P. moniliformis* immediately following application (A) and after two years of storage (B), for different exposure times



The speed of germination or primary root emergence depends on factors that regulate metabolism, water absorption and, consequently, biochemical reactions (Bewley *et al*., 2013; Godoi; Takaki, 2005). They can also be used as parameters to identify batches, as seeds with these characteristics become resistant to adverse conditions during their development in the field.

There was an increase in seedling length as a function of the time exposed to the plasma. Seedlings from seeds treated for 5 min had an average length of 5.20 cm seedling^{-1} for the shoots (Figure 4A) and 4.35 cm seedling^{-1} for the root system (Figure 4B). However, for the seeds submitted to the plasma and then stored, there was a reduction in seedling length, with an average of 2.17 and 1.99 cm seedling^{-1} for the shoots (Figure 4A) and root system (Figure 4B), respectively.

Figure 3 - Germination speed index in seedlings of *P. moniliformis* under the immediate effect of plasma application and after storage, for different exposure times



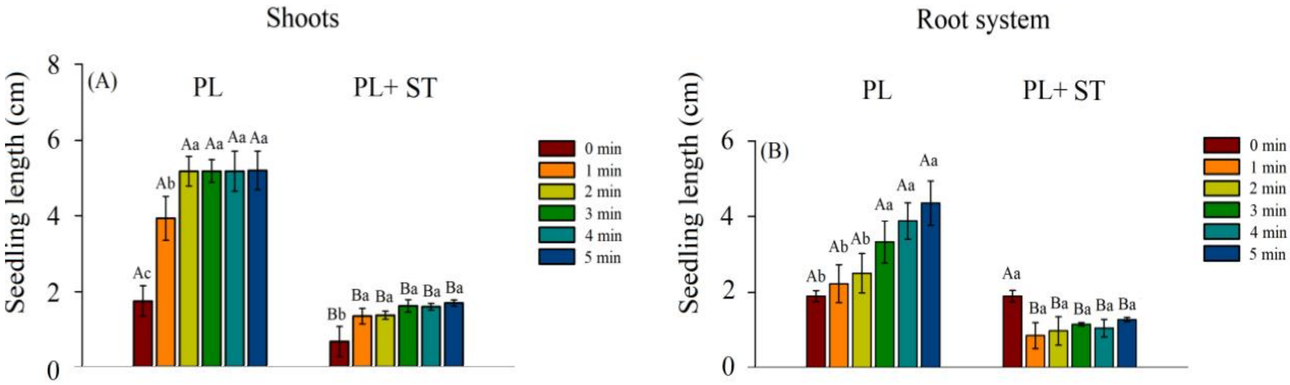
Seedlings from the untreated seeds (control) were smaller than those produced under any of the exposure conditions, averaging from 1.75 to 1.70 cm seedling^{-1} for the shoots and 1.99 to 1.27 cm seedling^{-1} for the root system (Figure 4).

There was a significant increase in seedling length after five minutes of plasma treatment, demonstrating the stimulative effects of the treatment in promoting initial growth. However, subsequent seedling growth in the seeds that were treated and stored was reduced, raising the possibility that the initial stimulative effect of the plasma may have been temporary due to the gradual degradation of the bioactive components during storage. In addition, the complex interactions between the plasma treatment, storage and environmental factors suggest that more research is needed to fully understand these results.

Corroborating the results of this study, Jiang and Dong (2018) found that plasma had a positive effect on germination and shoot growth in tomato seedlings (*Solanum lycopersicum*) from seeds treated with different concentrations of the ionic gas. Similar results were found by Mildaziene *et al*. (2018) in clover seeds (*Trifolium polymorphum Poir*) treated for 10 and 15 min, which presented a well-developed and branched root system.

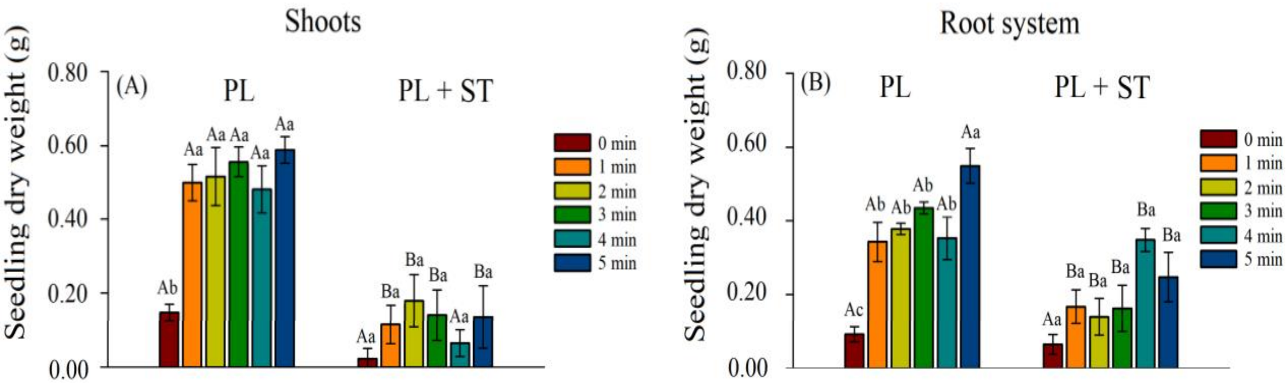
The effect of the plasma on the seeds was assessed by analysing the dry weight of the seedlings, which showed similar values to those obtained in this study for seedling length. In this case, the seeds that were treated and analysed immediately showed greater dry weight for each of the conditions under test compared to the control (Figure 5).

Figure 4 - Shoot length (A) and root length (B) in seedlings of *P. moniliformis*under for different plasma exposure times (0, 1, 2, 3, 4 and 5 minutes), evaluated immediately (PL) and after 24 months of storage (PL+ST)



Mean values followed by the same uppercase letters between exposure times and lowercase letters for storage do not differ statistically by Tukey's test ($P \leq 0.05$)

Figure 5 - Shoot dry weight (A) and root dry weight (B) in seedlings of *P. moniliformis* for different plasma exposure times (0, 1, 2, 3, 4 and 5 minutes), evaluated immediately (PL) and after 24 months of storage (PL+ ST)



Mean values followed by the same uppercase letters between exposure times and lowercase letters for storage do not differ statistically by Tukey's test ($P \leq 0.05$)

Different results from those seen in this study were found by Guragain *et al*. (2022), when the dry weight of radish seedlings (*Raphanus sativus*) in the control treatment was greater than from seeds treated for 1 to 5 minutes. However, it should be noted that the information obtained from seed vigour tests should not be interpreted individually, as other factors need to be considered, such as seedling length and germination (AOSA, 1983).

In some cases, there may be batches showing high germination, albeit made up of smaller-sized seedlings, and vice versa (Guedes *et al*., 2009). Depending on the purpose and the species used, the response to the effect of the plasma may vary according to the parameters being evaluated, requiring precise and individualised assessments.

CONCLUSIONS

1. Plasma is immediately effective in overcoming seed coat dormancy in seeds of *P. moniliformis* treated for five minutes;
2. After storage, the effect of the plasma was less than its immediate application in seeds of *P. moniliformis* for each of the conditions under test.

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