Colloids and Surfaces B: Biointerfaces 157 (2017) 280–285

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Protocols

Using atmospheric plasma to increase wettability, imbibition and germination of physically dormant seeds of *Mimosa Caesalpiniafolia*

A.R.M. da Silva [a], M.L. Farias [a], D.L.S. da Silva [a], J.O. Vitoriano [a], R.C. de Sousa [b], C. Alves-Junior [a,*]

[a] *LABPLASMA- Department of Exact and Natural Sciences, Federal Rural University of Semiarid, Mossoró, RN, CEP: 59625-900 Brazil,*
[b] *LCMM – Physics Department, Federal University of Maranhão São Luís, MA, CEP: 65080-805, Brazil*

ARTICLE INFO

Article history:
Received 22 January 2017
Received in revised form 22 May 2017
Accepted 25 May 2017
Available online 1 June 2017

Keywords:
Atmospheric plasma
DBD
Seed dormancy
Impermeable integument

ABSTRACT

In this study, we analyzed seed wettability as well as imbibition and germination after treatment with atmospheric pressure cold plasma (APCP) using dielectric barrier discharge (DBD) in seeds that have very low germination rates. To aid industrial applications, several seeds were simultaneously treated with plasma within a space between two coaxial glass tubes sandwiched by two metal mesh screens that produced high-voltage pulses at 17.5 kV with a frequency of 990 Hz. Three treatment times (3 min, 9 min and 15 min) as well as untreated seeds were used to conduct the wettability, imbibition and germination tests. The wettability and imbibition were found to be directly related to the treatment duration, but saturation of the imbibition was found for treatment durations greater than 9 min. Plasma treatment was also effective in improving germination, but shorter treatment duration presented greater germination. This apparent contradiction is explained by the cell damage caused by the increased exposure to plasma, as observed in other studies. The results suggest that there must be an optimal wettability and imbibition condition that ensures that excessive moisture does not harm the germination process.

1. Introduction

The large-scale cultivation of species such as Mimosa caesalpiniafolia Benth is very important for ecosystems and agricultural areas in maintaining soil fertility and restoring degraded areas [1,2]. However, due to integument dormancy, viability is very low (less than 10%), making the culture unfeasible. The methods used to overcome seed dormancy and to improve germination rate generally consist of increasing wetting and soaking [3].

Physical (scarification) and chemical (use of sulfuric acid and hot water) treatments are commonly used in agriculture and have high environmental costs [4]. Reduction in vigor, higher infection rate due to structural damage, increased probability of abnormality in seedling growth shoots and production of poor fitomass, in addition to residues resulting from chemical treatments, are some examples of these problems [5–7]. In recent years, there has been a growing number of agricultural applications of plasma produced by dielectric barrier discharge (DBD), especially related to the inactivation of microorganisms and overcoming seed dormancy [8–10]. Since seed tissue rehydration is essential for metabolic activities, which result in germination, plasma techniques must be able to modify the surface to increase seed soaking. The surface of Mimosa seeds has a complex structure that consists of an impermeable tegument (exotesta) containing small slits that communicate with mesotesta [11]. It has been reported that plasma species that are 'attenuated' by the coat do not have sufficient energy for the modification of the wettability of the internal sides of the biological surfaces [12]. However, several studies show that plasma can efficiently increase imbibition and germination [9,10]. The levels of GA3 hormone and mRNA expression (biochemical processes required for germination) of an amylolytic enzyme-related gene in seeds increased after treatment with high-voltage plasma pulses [13]. However, it is still unclear whether the seed viability can be changed when only the exotesta is modified by plasma. For most seeds, germination begins with a rapid initial water uptake that is higher on surfaces with more wettability. However, excessive moisture can decrease the absorption of oxygen and affect the germination process [14]. Strategies to enhance germination might involve wettability control on seed exotesta.

In this paper, we investigate the response with respect to wettability, imbibition and germination after treating Mimosa caesalpiniafolia seeds using different durations of DBD plasma treatment. To facilitate future industrial applications, several seeds were simultaneously treated with plasma in a space between two

* Corresponding author.
E-mail address: clodomiro.jr@hotmail.com (C. Alves-Junior).

http://dx.doi.org/10.1016/j.colsurfb.2017.05.063
0927-7765/

coaxial glass tubes sandwiched by two metal mesh screens that were connected to a power source.

2. Materials and methods

2.1. Seeds morpho-anatomy

Microstructures of the integument were analyzed using a scanning electron microscope and an optical microscope. For the analysis with the optical microscope, untreated seeds were soaked in a formalina-acetic acid alcohol (FAA) solution for 24 h, dehydrated in an ethanol process and embedded into paraffin blocks. The blocks were then cut into sections 10-μm thick with a rotary microtome. Histochemical testing was performed using toluidine blue dye to reveal lignin and/or cellulose [15]. The images were recorded with a digital camera attached to the optical microscope.

2.2. Plasma experimental apparatus

The experimental apparatus is illustrated in Fig. 1A. The source plasma was equipped with two coaxial glass tubes, which were externally and internally coated by a metal mesh screen, and each glass tube had a wall that was 1-mm thick. The metal mesh screen was connected to a high-voltage power source with a peak value of 17.5 kV that pulsed at a frequency of 990 Hz. The power density (p) was determined with the aid of the Lissajous figure area (S_L), which was obtained by an oscilloscope using a 1000:1 probe (Tektronix P6015A, Tektronix, Beaverton, USA), by Eq. (1) [16]:

$$p = \frac{P_d}{A} f = \frac{\int V(t)dQ(t)}{A} f = \frac{S_L f}{A} \quad (1)$$

where P_d is the dissipated electrical power; A is the area irradiated by plasma the area; S_L is the area under the curve of the Lissajous; and V, Q, and f are the voltage, charge and frequency of the circuit, respectively.

An optical fiber was used to transfer the light spectrum from the plasma to the CCD spectrometer (HR4000, Ocean Optics, Dunedin, FL, USA) – the spectral range is from 200 to 1000 nm – to measure the reactive species generated in the plasma.

2.3. Plasma treatment

The slightly elliptical seeds, which have an average diameter of 6.0 mm, were placed between the two glass tubes within the plasma reactor. For the plasma treatment, around 100 seeds were uniformly distributed between the coaxial tubes at a time. Under these conditions, the plasma filled the spaces between the two glass tubes and evenly coated all of the seeds' surfaces (Fig. 1B). The treatments were performed for durations of 3, 9 or 15 min, depending on the treatment group. After treatment, the seeds were stored in dissectors to test the wettability, imbibition and germination.

2.4. Wettability test

Wettability tests were conducted using the sessile drop technique to measure the apparent contact angle of distilled water droplets. It was measured on 3 seeds under each condition [17]. The test was performed by dripping 20 μl of distilled water once on each seed surface. The images were captured and recorded by a camera attached to a computer and processed to determine the contact angles using the Surftens® 3.5 program. Descriptive statistics (arithmetic mean and standard deviation) were calculated for the values of the contact angles formed by the liquid and the integument.

2.5. Imbibition test

The seeds were soaked in plastic Gerbex-type boxes (capacity of 250 ml, dimension 11.0 × 11.0 × 3,5 cm3). The imbibition test began by placing the seeds between two germination papers, where a proportion of 3.0 ml of water was added to each gram of paper. For each test, 40 seeds were divided into 4 replicates of 10 seeds. The seeds were removed after 0, 4, 12, 24 and 36 h and weighed; the percentage increase in seed mass variation was determined by Eq. (2):

$$\%Mass = \frac{m_f - m_i}{m_i} x100 \quad (2)$$

where m_f is the mass of the imbibed seeds, and m_i is the mass of the dry seeds.

The test was conducted in a BOD type of germination chamber, temperature controlled at 25 °C and a photoperiod of 8 h.

2.6. Germination test

The germination capacity test was also carried out in plastic Gerbox boxes filled with sterilized sand. Prior to sowing the seeds, each box was prepared with 400 g of sand and 60 ml of distilled water. In each test, 100 seeds were divided into 4 replicates of 25 seeds. The germination of the DBD plasma-treated seeds were compared to the untreated seeds after 12 days. The experimental design was entirely carried out at random.

The description of germination kinetics was obtained by fitting the experimental points to the Richard curve [18,19]. The Richards function (Yt), which has a variable inflection point, is represented by the Eq. (3):

$$Y_t = \frac{\alpha}{[1 + b + dx\exp(-cxt)]^{1/d}} \quad (3)$$

From this equation, the following parameters are obtained: Vi (viability), which is the total percentage of germination; Me (Median), which is the time of occurrence of 50% of the total germination. The Me parameter is very important for the description of the germination process and the initial growth of seedlings, as it distinguishes the behavior of the species or plant variety during germination. Qu indicates the dispersion of Me, and Sk (asymmetry) indicates the asymmetry in the frequency of distribution of the germination time, which represents the asymmetry of the Richards' function relative to the inflection point [19,20].

2.7. Statistical analysis

The experimental design was completely randomized (DIC) for the analysis of variance (ANOVA). The Tukey ($P < 0.05$) average comparison test was performed using Sisvar® software.

3. Results and discussion

3.1. Dissipated power and active plasma species

Integrating the Lissajous figure in Fig. 2 and considering the area irradiated by plasma (A), which is equal to 0.006 m^2, the dissipated power density for a frequency of 990 Hz can be obtained using Eq. (3):

$$p = \frac{1.07\times10^{-6}}{A} * 990 = 0.18 w/m^2 \quad (3)$$

The optical spectra of the plasma (Fig. 3) was obtained using an optical fiber in front of the coaxials tubes, which transmitted to an optical emission spectrometer (OES) diagnostic of plasma species. The main peaks result from the excitation of the nitrogen molecules (N2) present in the air, which are all from the second pos-