doi: 10.21829/myb.2019.2521665
Madera y **Bosques** vol. **25**, núm. **2**, e**2521665** Summer **2019**
Scientific papers

Laser biostimulation for improving seeds germinative capacity and seedlings growth of *Prosopis laevigata* and *Jacaranda mimosifolia*

Bioestimulación láser para mejorar capacidad germinativa de semillas y el crecimiento de plántulas de *Prosopis laevigata* y *Jacaranda mimosifolia*

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Abstract

"Jacaranda" (*Jacaranda mimosifolia*) and "mezquite" (*Prosopis laevigata*) are timber species that generally present problems with regard to the germination of their seeds in natural conditions, since they have a very hard and impermeable cover. Different treatments have been applied to improve the germinative response of both species with unfavorable results. This study aimed to know the effect of the pre-sowing laser biostimulation on germination and seedlings growth of these species. The seeds were biostimulated with a He-Ne laser (632 nm, 10 mW). Considering three replications of 50 seeds for each species, five irradiation treatments were applied (30 s, 60 s, 90 s, 120 s, 150 s) and control. The results for mezquite demonstrated that the highest number of germinated seeds (96%) was obtained with the treatments 90 s and 150 s; in contrast, the control seeds showed a lower germination percentage (16%). Additionally, the 30 s treatment produced a positive effect on the growth of the root, and it was different ($p < 0.05$) to the remaining treatments and the control. For *Jacaranda*, there were significant statistical differences ($p < 0.05$) between the control and the different treatments. 29% of seed germination occurs without any treatment. However, for 90 s and 120 s, germination is induced in a relatively high percent (97%-99%). For both species, the best treatment was 120 s, since higher values were recorded for all the morphological variables. It is concluded that laser biostimulation can produce beneficial effects on the germination of seeds and seedling growth and it could contribute to the propagation and conservation of these species.

Keywords: anatomical changes, biochemical characteristics, He-Ne laser, germination percentage.

Resumen

"Jacaranda" (*Jacaranda mimosifolia*) y "mezquite" (*Prosopis laevigata*) son especies que presentan problemas en la germinación de sus semillas en condiciones naturales, por tener una cubierta muy dura e impermeable. Se han aplicado diferentes tratamientos para mejorar la respuesta germinativa de ambas especies con resultados poco favorables. Este estudio tuvo como objetivo conocer el efecto de la bioestimulación láser en semillas sobre la germinación y el crecimiento de plántulas en estas especies. Se utilizó un láser He-Ne (632 nm, 10 mW). Con tres repeticiones de 50 semillas, se aplicaron cinco tratamientos de irradiación (30 s, 60 s, 90 s, 120 s, 150 s) y el control. Los resultados para el mezquite demostraron que el control tuvo el menor porcentaje de germinación (26%), y el mayor número de semillas germinadas (96%) fue para 90 s y 150 s de irradiación. El tratamiento de 30 s produjo un efecto positivo sobre el crecimiento de la raíz y fue significativamente diferente ($p < 0.05$) a los tratamientos restantes y al control. Para la jacaranda, se observaron diferencias significativas ($p < 0.05$) entre el control y los tratamientos, las semillas sin ningún tratamiento germinaron 29% y con los tratamientos de 90 s y 120 s la germinación fue de 97%-99%. Adicionalmente, destacó el efecto del tratamiento 120 s, ya que se registraron valores más altos para todas las variables morfológicas. Se concluye que la bioestimulación con láser puede mejorar el porcentaje de germinación y el crecimiento de las plántulas, por lo que podría contribuir significativamente a la propagación y conservación de estas especies.

Palabras clave: cambios anatómicos, características bioquímicas, láser He-Ne, porcentaje de germinación.

INTRODUCTION

Among physical and chemical methods to improve the effectiveness of germination, laser stimulation has shown positive effects on seeds germination and growth of seedlings of various species (Podleśny, Stochmal, Podleśna, & Misiak, 2012; Jamil *et al*., 2013; Prośba-Bialczyk *et al*., 2013). In general, a laser is a device that produces a beam of light with certain optical properties, like intensity, emission wavelength, beam divergence, etc. In plants, laser stimulation is a physical phenomenon based on the ability of cells to absorb and store radiant energy (Gladyszewska, 2011, Sacala, Demczuk, Grzyś, Prosba-Bialczyk, & Szajsner, 2012). The same phenomenon can be observed in the seeds, because they absorb the energy of light to subsequently transform it into chemical energy for use in the growth (Dinoev, Antonov, Stoyanov, & Georgieva, 2004; Chen, Yue, Wang, & Ling, 2005; Chen, Jia, & Yuen 2010; Dziwulska, Wilczec, & Ćwintal *et al*., 2006). Literature data claim that laser irradiation as a method of pregerminative stimulation of the seed has a positive effect on plant growth and metabolism of many species of commercial interest, as soybean, wheat, maize, radish, tomato, alfalfa, clover, carrots, pea and sugar beet (Rybiński, 2000; Aladjadjiyan, 2007; Benavides, Garnica, Hernández, Fuentes, & Ramírez, 2007; Sujak, Dziwulska-Hunek, & Kornazyński, 2009; Hernández-Aguilar *et al*., 2010; Gladyszewska, 2011, Sacala *et al*., 2012). A dose of energy with a red laser (He-Ne) can be used as pregerminative treatment for seeds. This stimulation will rise the energy potential of seeds and improve germination (Truchliński, Wesolowsky, Koper, & Dziamba, 2002; Gladyszewska, 2011). Also, laser irradiation might activate phytochrome which consequently modulates plant response as well as their ability to produce young plants more vigorous in the first stage of its development (Sacala *et al.,* 2012). According to Hernández-Aguilar *et al.* (2010), the basis of the stimulation mechanism in any plant physiological stage is the synergism between the polarized monochromatic laser beam and the photoreceptors. In this regard, there are three main classes of photoreceptors in plants: phytochromes, sensitive to the red and far-red region of the visible spectrum, cryptochromes in the blue and UV-A region and phototropins (Lariguet & Dunand, 2005; Torres, Huang, Chua, & Bolle, 2006).

A large number of forest species do not germinate because the testa or cover seminal is hard and prevents the entry of water (physical latency), and the seed does not germinate unless it is scarified. "Mezquite" (*Prosopis laevigata*) generally presents problems with regard to the germination of its seeds in natural conditions, since they have a very hard and impermeable cover that prevents the water from passing through, inhibiting in part the germination, which causes that cover to become a problem when trying to manage the seed for reproductive purposes (Rivas-Medina, González, Valencia, Sánchez, & Villanueva, 2005). Different methods have been used with the purpose to improve mezquite's seeds germination (D'Aubeterre, Principal, & García, 2002; Rivas-Medina *et al*., 2005; García-Aguilera, Martínez-Jaime, Torres, & Frías-Hernández, 2000; Pérez-Sánchez, Jurado, Chapa-Vargas, & Flores, 2011; Brandt, Lachmuth, Landsschultz, Hab, & Jensen, 2014). In the case of "jacaranda" (*Jacaranda mimosifolia*) a deciduous tree, the seeds, also have a hard testa and are inside a fruit or pod with a hard cover that when ripe is dehiscent and releases the seeds. However, the type of fruits and their conservation time affect the germination capacity of the seeds, according to Póvoa (2018), who observed germination results ranged from 11.3% (dark brown old fruits) to 93.5% (light brown, new fruits). Other methods of propagation of *Jacaranda mimosifolia* include the addition of GA_3 to immature seeds of *Jacaranda mimosifolia* (Miyajima *et al*., 2005) and thermal treatments to the seeds (Póvoa, 2018). Works on the application of laser and its effect on the germination of this species of *Prosopis* and other woody species including *Jacaranda mimosifolia* are lacking.

P. laevigata is a natural resource in the arid and semi-arid areas of the southern part of the USA and central-northern Mexico. *P. laevigata* not only help to retain water, fix nitrogen (Orozco-Villafuerte, Cruz-Sosa, Ponce-Alquicira & Vernon-Carter, 2003), and store CO_2 for long periods (Méndez, Turlan, Ríos, & Nájera, 2012), but also each part of the plant is used as a source for human and animal food. Its pods for example, are consumed fresh, ripe or dried building material (firewood, fodder, coal, manufacture of crafts). *P. laevigata* also has medicinal properties, its leaves are a source of bioactive phenolic compounds and nutraceutical ingredients with antioxidant capacity and cardioprotection potential (Azero & Andrade, 2006; García-Andrade *et al*., 2013; Rodríguez-Sauceda, Rojo-Martínez, Ramírez-Valverde, Martinez-Ruiz, Cong-Hermida, Medina-Torres, & Piña-Ruiz, 2014). This species has phytoremediation potential (Buendía-González, Orozco-Villafuerte, Cruz-Sosa, Barrera-Díaz, & Vernon-Carter, 2010), for wastewater treatment (Torres, Carpinteyro-Urban, & Vaca, 2012) and is also employed for charcoal production for domestic consumption and export (Rodríguez-Sauceda *et al*., 2014; Foroughbakhch *et al*., 2012, Orozco-Villafuerte *et al.,* 2003; Saucedo-Anaya *et al*., 2017). *Jacaranda mimosifolia* is an ornamental species, with medicinal applications (Food and Agriculture Organization of the United Nations [FAO], 2003 a, b; López-Franco, Goycoolea, Valdez, & Calderón, 2006; Palacios, 2006; Rojas, Ochoa, Ocampo, & Muñoz, 2006). *Jacaranda mimosifolia* is used as forest for its wood easy to work and good quality. Its wood is semi-hard, semi-heavy and yellowish-white with soft veining. It is also used for furniture manufacture, interiors of cars, coatings, general carpentry and carving sculptures. Also, the flowers from jacaranda and mezquite are important in the production of honey bee. *Prosopis laevigata*, for example, represents the most important source of pollen and nectar for pollinators from March to May in some semiarid regions where the trees may represent, 89.9% - 91.2% of the total of Fabaceae species (Valenzuela *et al*., 2015; Medina-Cuéllar, Tirado-González, Portillo-Vázquez, López-Santiago, & Franco-Olivares, 2018). Mexico is one of the main honey exporters of the world (Secretaría de Agricultura y Desarrollo Rural [Sagarpa], 2015; Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria [Senasica], 2015). On the other hand, *Jacaranda mimosifolia* is also on the list of important plant species for honey production in other regions of the world (Beyene & Hiwot, 2015).

Although the seeds of *Prosopis laevigata* are orthodox, they do not present latency, only hard seed coat dormancy, but once the seeds are devoid of the different layers that surround them, the germination appears immediately (Hong, Linington, & Ellis, 1996; Rodríguez-Sauceda *et al*., 2014). For this reason, *Prosopis laevigata* seeds usually have problems related to germination under natural conditions, due the presence of a hard and impermeable testa (seed coat) that prevents the passage of water and inhibits germination (Maldonado-Aguirre & De la Garza, 2000; Rivas-Medina *et al*., 2005). On the other hand, *Jacaranda mimosifolia* is a species propagated by seed (Socolowski & Massanori, 2004; Li, Zhou, Shi, & Gao, 2012), the seeds also have a hard testa and are inside a fruit or pod with a hard cover that when ripe is dehiscent and releases the seeds. Although their seeds are recalcitrant (germinate immediately), the survival of plants in natural conditions is low due to the dependence of open spaces (Wright *et al*., 2008). Nevertheless in this species, the speed and percentage of germination of the seeds greatly influences, whether they come from a fresh fruit or with some storage period (Póvoa, 2018). The response of their seeds to alternative treatments for germination stimulation it is unknown.

OBJECTIVES

The objective of this investigation was to evaluate the effect of applying He-Ne laser irradiation treatments on seeds of *Prosopis laevigata* (Humb. et Bonpl. ex Willd) M.C. Johnst, Fabaceae, and *Jacaranda mimosifolia* D. Don., Bignoniaceae to improve seed germination and shorten the seedling grow time for reforestation purposes.

MATERIALS AND METHODS

In this experiment, seeds of *Prosopis laevigata* and *Jacaranda mimosifolia* were collected from mature fruits of 3-6 individuals per species from wild and cultivated populations in Jalisco (21° 31´ N latitude, 101° 41´ W longitude; 1930 m asl), Mexico. The fruits of *Prosopis laevigata* are linear legumes 7 cm to 20 cm long by 8 mm to 15 mm wide, somewhat constricted between the seeds. Once mature, they have a yellowish-brown color, sometimes reddish. The seeds are oblong, compressed from 8 mm to 10 mm long, with a yellowish-white color, and a hard, waterproof cover. In *Jacaranda mimosifolia* the fruits are oblong flattened capsules, brown color, with a dry and hard cover. The fruit contains numerous, winged, hyaline or brownish seeds (Gilman & Watson, 1993; Mostafa, Eldahsan & Singab, 2014).To get the seeds, the dissection of the fruits was carried out. The seeds were washed with tap water to remove the remainder of the fruit pulp. Then they were placed in absorbent paper for drying, after this they were stored in paper envelopes under laboratory conditions at a temperature between 20-25 °C to maintain their viability.

For germination experiments, 15 days after collected fruits, the seeds were selected carefully discarding those that showed some visible damage in the testa. Then they were divided into groups of 50 seeds each; seeds with 10 mm ± 0.1 mm in length and 0.6 g ± 0.01 g in weight for *Prosopis laevigata*, while for *Jacaranda mimosifolia*, seeds with 7 mm ± 0.1 mm in width and 0.5 g ± 0.01 g in weight were used for germination experiments. A greater number of seeds must be included in germination experiments (International Seed Testing Association [ISTA], 2018); however, in this study it was decided to include three repetitions of 50 seeds each for treatment and control, because only this amount of seeds had the size and weight requirements. In this way, groups of seeds classified as viable, were subjected to treatment with different doses of He-Ne laser irradiation of low intensity (632 nm wavelength, 10 mW, CW) using a laser beam expanded to a size about 2.5 cm in diameter. Five irradiation treatments (30 s, 60 s, 90 s, 120 s, 150 s) and a control (without irradiation) were included.

To obtain the germination percentages, the record of germination was carried out through the count of germinated seeds for each treatment and for each species. The main effects of each treatment that were significant were analyzed with an ANOVA and a multiple comparison test of Tukey ($p < 0.05$). The statistical analysis was performed with statistical analysis SAS (SAS, 2002). In order to reveal the magnitude of species *vs* irradiation levels interaction, graphs with the interaction of morphological variables of seedlings and seed germination of all the treatments were obtained. For this purpose the free statistic software Multivariate Factor Analysis-Ungrouped Data version 1.2.1 (Wessa, 2018) was used.

The seedlings coming from both experiments (laser and control) were transferred to greenhouse, transplanted to pots with inert substrate (agrolita-vermiculite-ground potting, mixture 1:1:1), with irrigation (distilled water) to field capacity every two or three days during growth. Also, during initial growth of the seedlings (five days after germination and until 30 days age) a record of morphological characters (total height, root length, basal diameter of the seedling, length and diameter of hypocotyls, length and width of cotyledons) was carried out. For the measurement of morphological variables, a digital caliper Mitutoyo was used. It is known that transitory starch is synthesized in chloroplasts of photosynthetic tissues as one of the primary products of atmospheric CO_2 photosynthetic fixation (Weise, van Wijk, & Sharkey, 2011; Pessarakli, 2014). The presence of transitory starch in mesophyll tissue from the leaves, as a main feature to verify existence of photosynthetic activity was analyzed by histochemical tests (López-Curto, Márquez-Guzmán, & Murguía-Sánchez, 2005). A microscope adapted to an image analyzer IMAGE - Pro Plus version 6.1 was used for the observation of starch granules.

RESULTS

Significant statistical differences ($p < 0.05$), were observed for germination percentages in both species. For *Prosopis laevigata*, the obtained experimental data demonstrated that the highest percentage of germinated seeds (96%) was obtained from treated seeds with 90 s and 150 s as compared with the control showed a lower germination percentage (26%). The percentage of germinated seeds between treatments and control showed significant statistical differences ($p < 0.05$), except between treatments

30 s, 60 s, 90 s and 150 s (Fig. 1). The application of different ($p < 0.05$) from the remaining treatments and control (Table 1). The implementation of treatments 60 s and 120 s turned out to be better, they produced the highest values for length and width of cotyledon (8.1 mm and 7.5 mm, respectively) (Table 1). Seedlings from irradiated treatment 30 s produced a stimulatory effect positive on the growth of the root (54.2 mm, length), which is significantly seeds showed a normal development in the early stage of its growth (Fig. 2A and 2C). Similarly, the presence of starch grains in the mesophyll of the leaves could demonstrate that the cells carried out the photosynthetic function (Fig. 3B).

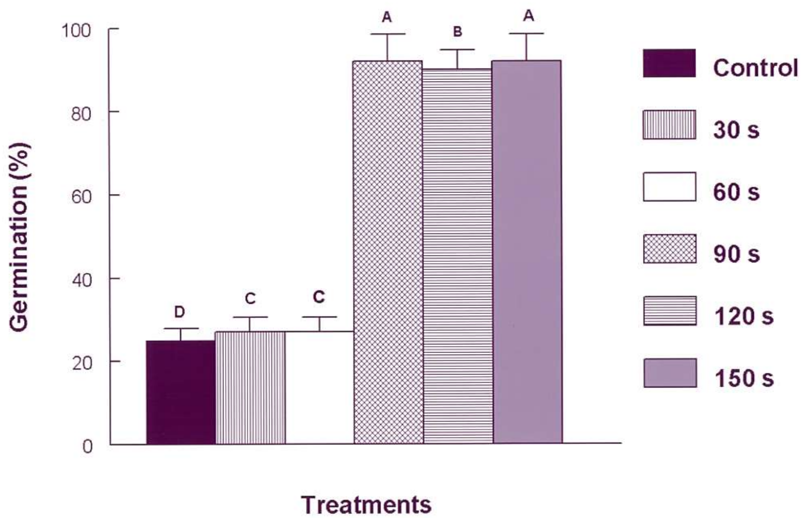

FIGURE 1. Germination percentage for *Prosopis laevigata* seeds biostimulated with different He-Ne laser treatments.
The bars represent the mean ± standard deviation. Different letters on the bars indicate significant statistical differences (Tukey, $p < 0.005$).

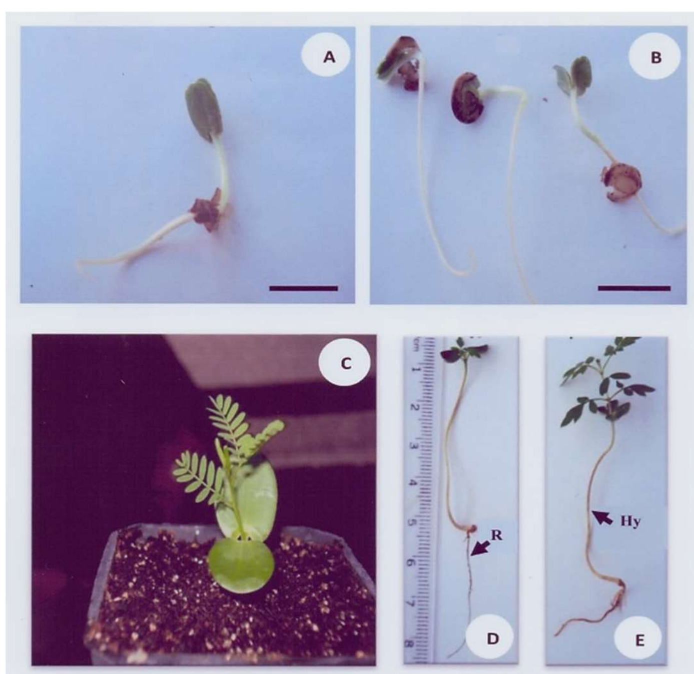

FIGURE 2. Seedlings of *Prosopis laevigata* (A and C) and *Jacaranda mimosifolia* in the early stage of its growth; seedling from not irradiated seed (D); B and E, seedlings from irradiated seed (60 s of irradiation).
Scale: 1 cm. Hy, hypocotyl; R, root.

TABLE 1. Morphological characters of *Prosopis laevigata* seedlings registered with the application of different laser He-Ne treatments and the control (without irradiation).

Treatments	*Root Length*	*Hypocotyl Diameter*	*Hypocotyl Length*	*Cotyledon Length*	*Cotyledon Width*
Control	21.3 ± 0.005 F	0.42 ± 0.006 E	26.2 ± 0.006 F	5.3 ± 0.003 E	3.6 ± 0.005 F
30 s	54.2 ± 0.005 A	0.51 ± 0.003 D	31.5 ± 0.003 E	5.3 ± 0.007 E	6.7 ± 0.005 E
60 s	39.0 ± 0.031 B	0.60 ± 0.006 B	37.5 ± 0.025 B	8.1 ± 0.006 A	7.4 ± 0.003 B
90 s	22.6 ± 0.003 D	0.52 ± 0.003 D	33.7 ± 0.009 D	7.5 ± 0.003 C	7.0 ± 0.009 D
120 s	28.7 ± 0.003 C	0.64 ± 0.003 A	42.2 ± 0.009 A	7.2 ± 0.006 D	7.5 ± 0.006 A
150 s	22.2 ± 0.005 E	0.59 ± 0.003 C	35.0 ± 0.020 C	7.8 ± 0.003 B	7.2 ± 0.003 C

*Dimensions are shown in mm. Significant statistical differences (Tukey, $p < 0.05$) in each column are indicated with different letters.

In the case of *Jacaranda mimosifolia*, there were significant statistical differences ($p < 0.05$) between the control and the different treatments. The obtained experimental data (Fig. 4), showed that 29% of seed germination occurs without any treatment. No significant statistical differences were observed between treatments 90 s and 120 s of irradiation; the same come about when treatments 60 s and 150 s are compared (Fig. 4). However, high percentage (97% - 99%). Also, as shown in table 2, the application of He-Ne laser produced a beneficial effect on with treatments 90 s and 120 s, germination is induced in a growth of seedlings. For example, the 120 s treatment had a positive effect on morphological characters, which showed, with respect to the control, an increase of root length (45.9 mm), hypocotyl diameter (0.64 mm), hypocotyl length (40.1 mm), and cotyledon size. Another effective treatment was the 60 s; in this treatment, the seedlings developed a root with 41.8 mm in length, as well as a hypocotyl of greater length and diameter (Table 2). In addition, with the implementation of this treatment the seedlings of *J. mimosifolia* showed a greater development and a highest number of leaves (Fig. 2D). In relation to the anatomical characteristics it was also observed that the leaf carried out the normal photosynthesis role as is the case with control seedlings, which is demonstrated by the presence of starch granules present in the mesophyll (Figs. 3C and 3D).

To understand the relationship between laser treatments *versus* morphological characteristics, a factor analysis has been used. Factor analysis is one of the statistical techniques that are effective to visualize an experimental behavior reducing the size of data. Factors analysis was performed based on morphological characters of tables 1 and 2. The two first factors were selected for the classification of the data (Fig. 5). For these two factors the cumulative variances were 93% and 95% for *Prosopis laevigata* and *Jacaranda mimosifolia*, respectively. Based on the results of factor analysis depicted in figure 5, we observe that all laser treatments improved the morphological characteristics for both species in a similar way with respect to the control; however, the treatment of 120 s was the one that gave the best results taking into account the set of all the morphological characters.

DISCUSSION

It is generally accepted that the germination process is sensitive to irradiation with various wavelengths of visible and infrared light, for the latter case, for example, it has to be mentioned that the red light could act on phytochrome system (photoreceptor) which promotes germination (Shichijo, Kazuya, Osamu, & Tohru, 2001). Both, the

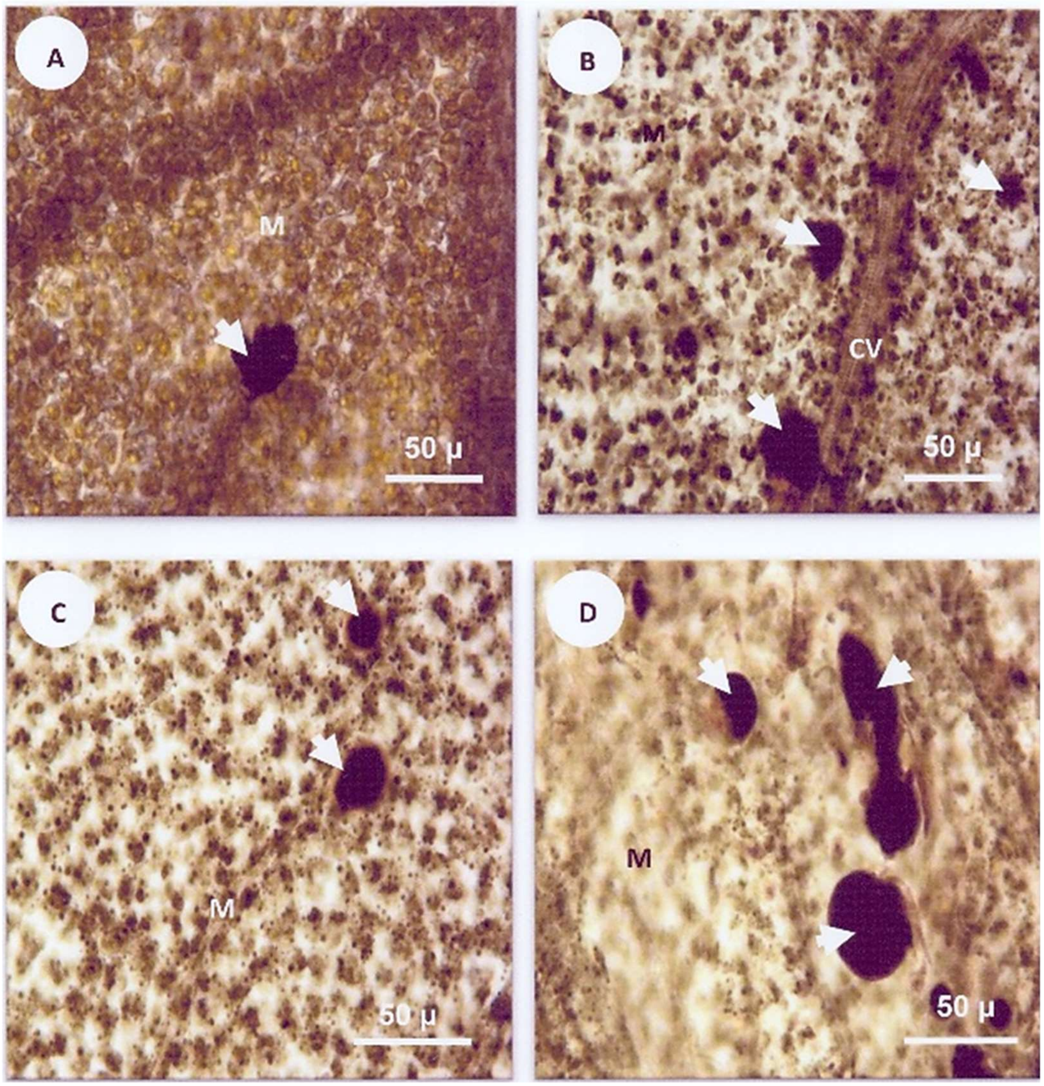

FIGURE 3. Presence of starch grains in the mesophyll of the leaves of *Prosopis laevigata* (A, not irradiated; B, irradiated), and *Jacaranda mimosifolia* (C, without irradiation; D, irradiated).

breaking of dormancy and germination stimulation with laser treatments have focused on several cereal grains and vegetables seeds, experimental evidence suggests that there are significant positive effects that improve the quality of plant products obtained from these irradiated seeds.

In the species studied, the beneficial effect of He-Ne laser irradiation can be expressed as an increase in the germination percentage of seeds, and seedlings of greater height when compared with the control. In other investigations it has been observed that the stimulation effect depends on laser wavelength (λ, in nanomilimeters), irradiation time interval (t, in seconds), irradiation dose (D, J/cm2), in addition to the seed characteristics and the requirement to soak them in water (imbibition). According to Aladjadjiyan (2007) and Hernández-Aguilar *et al.* (2008), the stimulatory effect is due to a further increase in the seed energy which is called bioplasm; therefore, raising the energy potential of this bioplasm raises the effect of stimulation for the seed to germinate (Truchliński *et al.*, 2002; Jamil *et al.*, 2013). In this way, it is possible to use red light (Helium-Neon) laser irradiation as pregerminative treatments of seeds to improve the germination capacity and strengthen the vigor of young plants or seedlings in the early stages of development since plants react positively

TABLE 2. Morphological characters of *Jacaranda mimosifolia* seedlings registered with the application of different laser He-Ne treatments and the control (without irradiation).

Treatments	*Root Length*	*Hypocotyl Diameter*	*Hypocotyl Length*	*Cotyledon Length*	*Cotyledon Width*
Control	34.2 ± 0.006 F	0.50 ± 0.006 E	28.4 ± 0.006 F	5.40 ± 0.003 C	5.0 ± 0.003 C
30 s	36.5 ± 0.007 D	0.60 ± 0.003 C	30.4 ± 0.006 E	5.25 ± 0.003 E	4.8 ± 0.006 E
60 s	41.8 ± 0.006 B	0.61 ± 0.006 B	38.6 ± 0.003 B	5.45 ± 0.003 B	5.0 ± 0.003 B
90 s	36.0 ± 0.003 E	0.55 ± 0.003 D	34.8 ± 0.003 D	5.35 ± 0.003 D	4.9 ± 0.003 D
120 s	45.9 ± 0.006 A	0.64 ± 0.003 A	40.1 ± 0.003 A	5.62 ± 0.003 A	5.2 ± 0.003 A
150 s	40.1 ± 0.003 C	0.59 ± 0.003 C	36.0 ± 0.003 C	5.20 ± 0.003 F	4.8 ± 0.003 E

*Dimensions are shown in mm. Significant statistical differences (Tukey, $p<0.05$) in each column are indicated with different letters.

TABLE 2. Morphological characters of *Jacaranda mimosifolia* seedlings registered with the application of different laser He-Ne treatments and the control (without irradiation).

Treatments	*Root Length*	*Hypocotyl Diameter*	*Hypocotyl Length*	*Cotyledon Length*	*Cotyledon Width*
Control	34.2 ± 0.006 F	0.50 ± 0.006 E	28.4 ± 0.006 F	5.40 ± 0.003 C	5.0 ± 0.003 C
30 s	36.5 ± 0.007 D	0.60 ± 0.003 C	30.4 ± 0.006 E	5.25 ± 0.003 E	4.8 ± 0.006 E
60 s	41.8 ± 0.006 B	0.61 ± 0.006 B	38.6 ± 0.003 B	5.45 ± 0.003 B	5.0 ± 0.003 B
90 s	36.0 ± 0.003 E	0.55 ± 0.003 D	34.8 ± 0.003 D	5.35 ± 0.003 D	4.9 ± 0.003 D
120 s	45.9 ± 0.006 A	0.64 ± 0.003 A	40.1 ± 0.003 A	5.62 ± 0.003 A	5.2 ± 0.003 A
150 s	40.1 ± 0.003 C	0.59 ± 0.003 C	36.0 ± 0.003 C	5.20 ± 0.003 F	4.8 ± 0.003 E

*Dimensions are shown in mm. Significant statistical differences (Tukey, $p<0.05$) in each column are indicated with different letters.

toward the light irradiation at wavelengths of 630 nm - 650 nm (Truchliński *et al*., 2002; Hernández-Aguilar *et al*., 2010). In other plant species (*Ricinus communis*), Helium Neon (He-Ne) laser light improved growth and decreased osmotic potential followed by increasing relative water content and help plants to complete its life cycle in comparison with untreated plants (Sami, Sharbat, Bedour, & Aly, 2014).

In a study of the effect of different doses of laser irradiation obtained from different powers (1 mW, 5 mW, 10 mW, and 15 mW) and exposure times (1 min, 5 min, 10 min and 15 min), in the germination of seeds from grass called "kudzu" (*Pueraria phaseoloides*), González, Fortes and Herrera (2008) have noted that the power and irradiation time exert different effects on the seeds germination; for example, there was an increase in the germination (40% up to 63%) when 1 mW laser power was used. For seeds of radish (*Raphanus sativus*) and spring barley (*Hordeum vulgare* L.), He-Ne laser irradiation (λ = 632.8 nm, and 5 mWcm^{-2}) can increase the final percentage of germination (FGP) Koper, Ćwintal, & Kornillowicz-Kowalska, 2005; Dziwulska *et al*., 2006; Kareem, El Tobgy, Osman, & El Sherbini, 2009; Perveen *et al*., 2011; Podleśny *et al.,* 2012; Sacala *et al*., 2012; Jamil *et al.,* 2013).

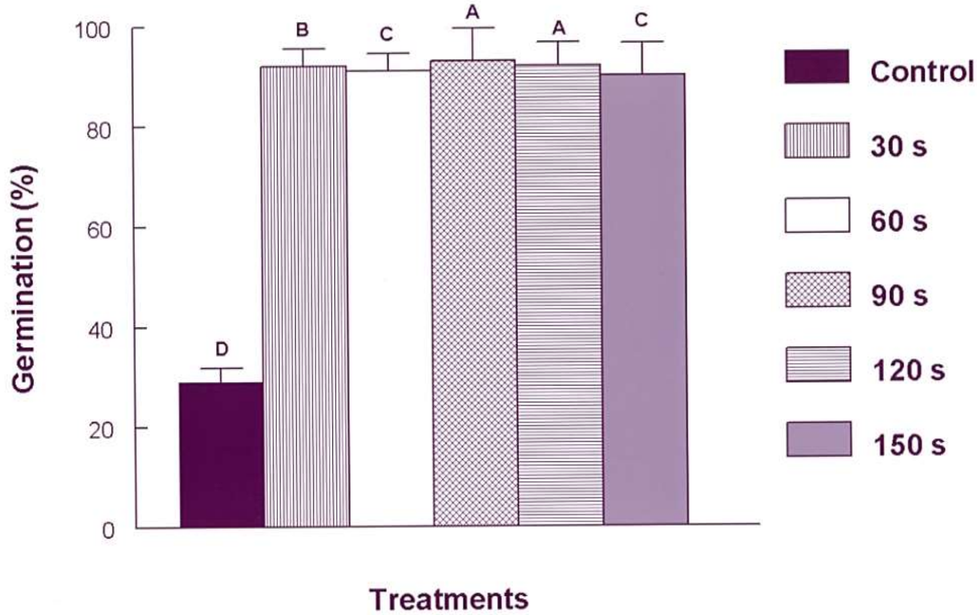

FIGURE 4. Germination percentage for *Jacaranda mimosifolia* seeds biostimulated with different He-Ne laser treatments.
The bars represent the mean ± standard deviation. Different letters on the bars indicate significant statistical differences (Tukey, $p < 0.005$).

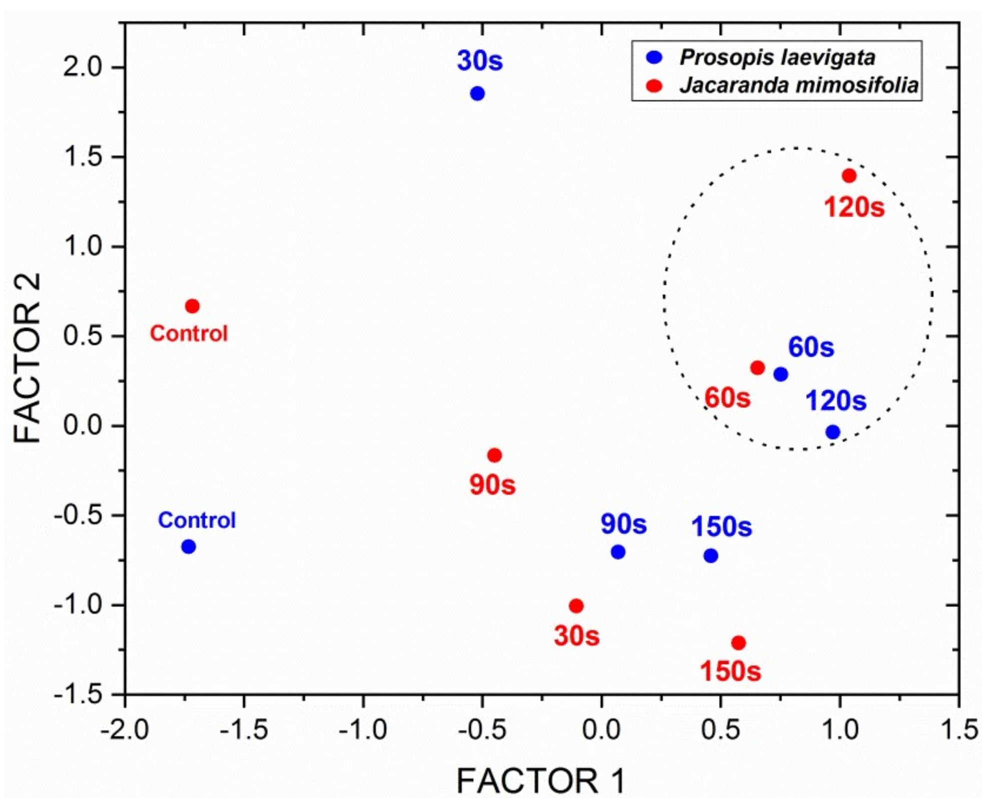

FIGURE 5. Factorial analysis of morphological characters for *Prosopis laevigata* (blue dots) and *Jacaranda mimosifolia* (red dots) corresponding to data shown in tables 1 and 2.

The biostimulation with He-Ne laser in addition to improving the germinative response of seeds and the growth of seedlings, has a beneficial influence on various biochemical processes in the plant (Abu-Elsaoud Abdelghafar, & Tuleukhanov, 2013; Taie, Lobna, Metwally, & Fathy, 2014, Abbas *et al*., 2017). According to Chen *et al.* (2005) for example, He-Ne laser pretreatment can improve the inner energy of seeds, lead to an enhancement of cotyledon enzymes and speed up the metabolism of the cell, significantly increased the cycles of cell division (mitosis) which results in an increase in the length of the plant organs during the early growth. Chen *et al.* (2005) studied the influence of laser irradiation on the thermodynamic and physiochemical parameters of seeds, and seedlings growth of medicinal plant *Isatis indogotica*, using an He-Ne laser (632.8 nm wavelength, 5.23 $mWmm^{-2}$ intensity), laser treatment had great influence with significant increase on pyruvic acid concentration, soluble proteins and saccharides in seedlings. In our investigation the presence of transitory starch was observed in the leaves of *Prosopis laevigata* and *Jacaranda mimosifolia* seedlings from irradiated seeds. It is known that transitory starch is synthesized in chloroplasts of photosynthetic tissues as one of the primary products of atmospheric CO_2 photosynthetic fixation. This type of starch accumulates in the form of granules insoluble by chloroplast during the day, granules that are degraded during the night ensuring a constant availability of sugars to all the plant. The correct regulation of the synthesis and degradation of starch is necessary for normal growth in a light-dark photoperiod. Also the synthesis and degradation of transitory starch affects various functions in the plant: flowering time, to the opening and closing of stomata and the maintenance of the photosynthetic rate (Pessarakli, 2014).

On the other hand, laser irradiation also has a beneficial effect by inducing biochemical changes of protection to plants when they are subjected to a certain type of stress or diseases (Starzycki, Rybiński, Starzycka, & Pszczola*,* 2005; Jia & Duan, 2013). For seedlings of *Prosopis* species which grow in arid and semiarid environments with high levels of solar radiation (Pérez-Sánchez *et al*., 2011), saline conditions, osmotic stress or contamination by heavy metals, treatment with He-Ne laser may result in a significant protective effect of damage to tissues as has been established to other plant species (Chen, 2010; Yang, Han, & Sun, 2012; Gao, Li, & Han.*,* 2015; Qiu *et al*., 2007, 2008, 2013). So, in future, another important research could be to assess whether it is possible to have this protective effect generated by this method of laser irradiation, including the seeds and seedlings in species of this genus in particular, whose habitat is extreme and strongly limits the recruitment of adults in the field. In addition, the mezquite is affected by an overexploitation process (Espinosa, 2006), therefore, implementing efficient methods for its propagation by seed, could contribute to the reestablishment of its populations and avoid, in turn, the deterioration of the ecosystems (García-Sánchez *et al*., 2012). In the case of *Jacaranda mimosifolia*, there is no information about the factors that affect their populations; however, since this species is characterized by having medicinal properties (Mostafa *et al.,* 2014), the application of laser irradiation as a pre-germinative treatment could be of great relevance for its propagation and use.

CONCLUSIONS

He-Ne laser treatments on seeds of *Prosopis laevigata* and *Jacaranda mimosifolia* had a positive effect on seed germination and morphological characters of seedlings. The greatest proportion of transitory starch demonstrated histochemically was observed in seedlings from irradiated seeds. The increased starch content may be related to its degradation for glucose production as energy source for the various metabolic reactions that take place during early growth of these species. The factorial analysis data processing showed that, independently of the laser treatment, germination percentages and morphological characters were improved, where the 120 s treatment, in general, showed the best results for both species. Although anatomical and biochemical changes in the seeds were not analyzed, it is highly probable that He-Ne laser irradiation had a significant influence on enzyme activities and acceleration in enzyme-mediated reactions, enhancing the biological activity and thereby causing enhanced entropy and internal energy of the seeds during germination, and as consequence an enhancement in growth of *Jacaranda mimosifolia* and *Prosopis laevigata* seedlings; nevertheless, further studies are required to make definite conclusions about this topic. The results show that this laser treatment may contribute significantly to the conservation and propagation of these species by the germination capacity and seedling growth improvement.

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Received: 10 November 2017

Accepted: 15 November 2018

Published: 19 August 2019

This paper must be cited as:

Costilla-Hermosillo, M. G., Ortiz-Morales, M., Loza-Cornejo, S., Frausto-Reyes, C., & Aly M, S. (2019). Laser biostimulation for improving seeds germinative capacity and seedlings growth of *Prosopis laevigata* and *Jacaranda mimosifolia*. *Madera y Bosques*, *25*(2), e2521665. doi: 10.21829/myb.2019.2521665