ΦYTON
REVISTA INTERNACIONAL DE BOTÁNICA EXPERIMENTAL
INTERNATIONAL JOURNAL OF EXPERIMENTAL BOTANY
FUNDACION ROMULO RAGGIO
Gaspar Campos 861, 1638 Vicente López (BA), Argentina
www.revistaphyton.fund-romuloraggio.org.ar

Morphology, viability and germination of candelilla seeds (*Euphorbia antisyphilitica* Zucc.)

Morfología, viabilidad y germinación de semillas de candelilla (*Euphorbia antisyphilitica* Zucc.)

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Abstract. Candelilla (*Euphorbia antisyphilitica* Zucc.) is a native plant in the desert regions of northern Mexico and the southern United States. The primary commercial use of this plant is for wax production. This study assessed the morphology, viability, and the effects of temperature in combination with chemical and mechanical scarification on the germination of candelilla seeds. Seeds were collected in four localities in northeastern Mexico. The seeds were predominantly pyriform with foveolate surfaces and brown in color; on average, the seeds measured 2.92 ± 0.26, 1.91 ± 0.22, and 1.50 ± 0.41 mm in length, width, and thickness, respectively. The average weight of 100 seeds was 0.32 ± 0.12 g. Seed viability showed a direct relationship with the color of the seed coat (thereby indicating the maturity level of the seed), with a viability of up to 86% in brown seeds, 52% in amber seeds, and 22% in gray seeds. Only brown seeds were used to assess germination after applying mechanical (sandpaper) and chemical (H_2SO_4) scarification, as well as two temperature (28 and 32 °C) treatments. The average germination rate was greater at 28 °C than at 32 °C for all treatments. The chemical scarification (at 28 °C) of seeds from the Lomas de Icamole locality showed the highest rate of germination (60%).

Keywords: *Euphorbia antisyphilitica*; Candelilla; Seed; Scarification; Germination.

Resumen. La candelilla (*Euphorbia antisyphilitica* Zucc.) es una planta nativa de áreas desérticas del norte de México y sur de Estados Unidos. La utilización de la planta para la obtención de cera es su principal uso comercial. El presente estudio evaluó la morfología, viabilidad y germinación de las semillas de candelilla, considerando para esto último el efecto de la temperatura y la escarificación química y mecánica. Las semillas se colectaron en cuatro localidades del noreste de México. La semilla es predominantemente de forma piriforme, superficie foveolada y coloración café, con medidas promedio de 2,92 ± 0,26; 1,91 ± 0,22 y 1,50 ± 0,41 mm para largo, ancho y grosor respectivamente. El peso promedio de 100 semillas fue de 0,32 ± 0,12 g. La viabilidad de la semilla mostró una relación directa con el color de la testa (estado de madurez de la semilla), alcanzando valores promedio de 86% en semillas cafés, 52% en semillas ámbar y 22% en semillas grises. Para evaluar la germinación se utilizaron semillas cafés, aplicando tratamientos de escarificación mecánica (papel lija), química (H_2SO_4) y testigo con dos temperaturas (28 y 32 °C). En todos los tratamientos la germinación promedio fue mayor en la temperatura de 28 °C. El tratamiento con mayor porcentaje de germinación (60%) fue la escarificación química a 28 °C en la localidad Lomas de Icamole.

Palabras clave: *Euphorbia antisyphilitica*; Candelilla; Semilla; Escarificación; Germinación.

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Recibido Received 25.IX.2012. Aceptado / Accepted 21.XI.2012.

INTRODUCTION

Candelilla (*Euphorbia antisyphilitica* Zucc.) is a perennial shrub native to arid areas of northern Mexico and the southern United States where the main limiting factor for plant development is precipitation. As an adaptive response to extreme drought conditions, this species produces wax, which is deposited on the stem surface and prevents the loss of water and excess caloric energy (Scora et al., 1995).

This wax has unique properties and is therefore in high demand in international markets for use in cosmetics, footwear wax, electric circuits, medication, paint, fruit covering, insulation, chewing gum, soap, balm, candles, disposable containers, computer cables, thermal paper, and dental models, among other applications (Hagenmaier, 2000; Tejeda et al., 2000; Cervantes, 2002; Ascacio Valdés et al., 2010).

The annual candelilla wax demand in the world market is approximately 3000 tons (Barsch, 2004). Because the wax constitutes 1.5 to 2.5% of the total dry weight of the plant (Schneider, 2009), approximately 150000 tons of plants must be collected each year, which results in a considerable pressure on candelilla populations.

Candelilla constitutes the main economic activity for many rural communities in northern Mexico (Canales et al., 2006), and this plant has been exploited for many decades. However, candelilla is currently produced in a non-sustainable manner because it is manually collected from wild populations by extracting the entire plant, which has caused a marked decrease in the populations of this resource (Barsch, 2004; Villa-Castorena et al., 2010) and the displacement of harvesters to more distant sites. Consequently, there is an urgent need for the implementation of strategies for reforestation and the sustainable use of this resource.

For the above reasons, and with the purpose of developing techniques that facilitate the large-scale reproduction of this species, the morphological variation in candelilla seeds from four localities in northern Mexico was evaluated in this study. In addition, we examined the viability of the seeds, as well as the effects of temperature and the application of chemical and mechanical scarification on the germination of this species.

MATERIALS AND METHODS

Fruit collection. Candelilla (*Euphorbia antisyphilitica* Zucc.) fruits were collected in four localities of northeastern Mexico where the plant is abundant under wild conditions: (1) Las Morenas [UTM (Universal Transverse Mercator) 735528, 2946478; 957 m.a.s.l. (meters above sea level)]; (2) Santa Teresa de Sofía (UTM 767637, 2951636; 825 m.a.s.l.); (3) Las Marías (UTM 724334, 3017215; 1277 m.a.s.l.); and (4) Lomas de Icamole (UTM 327788, 2868616; 659 m.a.s.l.). The first three localities are located in the state of Coahuila, and the fourth is found in the state of Nuevo León.

At each location, plants with ripe fruits were selected according to a systematic sampling design. Fruits were manually collected and placed into paper bags, which were labeled with information regarding the plant and the site. The fruit collection was performed in summer and fall when the flowering and fruit production of Candelilla occur. These are also the months with the highest precipitation (Flores-López, 1995).

Seed extraction. In the laboratory, the fruits were stored in plastic containers with bottom surfaces that absorbed humidity (blotting paper), and mesh lids that ensured air circulation while preventing the seeds from coming out of the containers when the fruits (explosively) opened. After the seeds were released from the fruits (approximately one week later), they were separated from the remainder of the fruits by passing all the material through a 2.5-mm sieve (larger than the size of the seeds) such that the fruit remains were captured by it. The collected seeds were stored in a cool and dry environment until they were analyzed.

Morphological characterization of the seeds. A total of 100 seeds per locality were measured using a dissecting microscope equipped with an ocular graduated at a scale of 1 cm and 40x magnification. The measured morphological variables included width (mm), length (mm), thickness (mm), shape, surface, and color. To measure weight, 30 replicates of 100 seeds each were randomly selected from each locality, and were weighed (g) using an OHAUS analytical balance.

Determination of viability. Because the color of a seed can indicate its maturity level (García-García, 1988; Peña-Contreras, 1998), seeds from each locality were classified into three groups according to color: A (brown), B (amber), and C (gray). Seed viability was determined using the tetrazolium test (ISTA, 1985). For this test, three replicates of 30 seeds each were randomly chosen from each group (color) of seeds; these replicates were first hydrated with distilled water for 12 h and then cut transversally through the seed coat to expose the embryo. After this procedure, seeds from each replicate were placed into 13 x 100 mm test tubes with 2 mL of tetrazolium chloride (2, 3,5-triphenyltetrazolium chloride) (Sigma-Aldrich™, St. Louis MO USA) at a concentration of 0.25 mg/L, and were incubated for 48 h at 24 °C. The interpretation of the test was based on the topographic pattern of the tetrazolium test for Candelilla (Flores-López, 1995). The percentage of viable seeds was determined using the following equation:

% viability = [(Number of colored seeds) / (Total number of seeds)] X 100

Pre-germination treatments. Prior to the germination trial, various treatments were assessed for the chemical and mechanical scarification and the disinfection of seeds, to select those treatments that allowed a higher rate of germination. The following treatments were evaluated:

Chemical scarification. Treatments with sulfuric acid (H_2SO_4) and nitric acid (HNO_3) were assessed, with concentrations varying from 5 to 40%, for a total of 12 treatments of 3 min each to avoid potential damage to the embryo.

Mechanical scarification. Mechanical scarification was assessed for two durations of treatment (2 and 5 min) with sandpaper (fine grain, trademark Fandeli key FIN 150).

Seed disinfection. A total of 8 disinfection treatments were assessed using 4% sodium hypochlorite (NaOCl; Cloralex®) diluted to 5, 10, 15, 20, 25, 30, 40, and 50% v/v with two treatment durations (3 and 5 min).

Germination trial. A complete randomized block design with three replicates was applied for the pre-germination treatments of seeds where the blocks represented localities. The trial evaluated the germination at two temperatures (28 and 32 °C) using two treatments (chemical and mechanical scarification) and a control. Each treatment had three replicates of 10 seeds each. Chemical scarification was performed by submerging seeds in 10% sulfuric acid for 2 min (the treatment was selected based on the preliminary trial), and the mechanical scarification was performed by rubbing the seeds with sandpaper for 2 min (the time selected based on the preliminary trial). After the treatments were applied, seed disinfection was performed using NaOCl at 4% diluted to 20% v/v for 5 min (the time and concentration were selected based on the preliminary trials); thereafter, the seeds were washed three times using distilled water. The seeds were placed into sterile Petri dishes and located in a bioclimatic chamber where germination was evaluated for 20 days. The percent germination (PG) was calculated using the following formula:

PG = [(Number of germinated seeds) / (Number of sowed seeds)] x 100

Statistical analysis. To verify significant differences in germination rates among treatments and localities, we used a two way analysis of variance (ANOVA) with a significant level of $p \leq 0.05$ together with a Tukey range test (Zar, 2010). The weight, length, and thickness variables as well as the variability of groups A, B, and C were analyzed using ANOVA ($p \leq 0.05$). The seed shape, color, and surface were compared using a Kruskal-Wallis test ($p \leq 0.05$). A linear regression model was applied to determine relationship between seed weight and the germination rate (Zar, 2010). Statistical analyses were performed using the software SPSS version 15.0.

RESULTS

Morphological characterization of the seeds.

Seed shape. The seeds in the studied localities were predominantly pyriform, including between 97 and 96% of the seeds from the Santa Teresa de Sofía and Lomas de Icamole localities, respectively. In Las Morenas and Las Marías, the percentages of pyriform seeds were 53% and 80%, respectively, whereas 3 to 12% and 5 to 17% were botuliform and ellipsoid seeds, respectively. Round seeds (24%) were found only in Las Morenas. A Kruskal-Wallis test showed a highly significant difference ($p \leq 0.01$) among localities with respect to seed shape.

Seed surface. Two types of surfaces were identified: foveolate and smooth. Foveolate surfaces were present in all localities up to a 100% in Las Morenas and Santa Teresa de Sofía, whereas smooth surfaces were only present in seeds from Lomas de Icamole and Las Marías at frequencies of 9 and 2%, respectively. The statistical analysis revealed significant differences among the localities ($p \leq 0.01$).

Seed color. Three seed colors, brown, amber, and gray, were present in the four localities at significantly different frequencies ($p \leq 0.01$). 1 Brown was the predominant color and it was present in at least 60% of the seeds in Las Marías, and up to 90% of the seeds in Santa Teresa de Sofía. Amber seeds were found at lower frequencies, with a maximum of 22% in Las Marías. Gray seeds were the least abundant in the four localities and were found in less than 1% of the seeds in Las Morenas, and up to 12% of the seeds in Las Marías.

Seed weight. The overall mean weight of 100 seeds was 0.32 ± 0.12 g. The greatest value (0.43 ± 0.07 g) was recorded in seeds from Santa Teresa de Sofía, and the lowest value (0.20

Table 1. Mean values ± standard deviation and Tukey test results from candelilla seeds morphometrical parameters.
Tabla 1. Valores promedio ± desviación estándar, y prueba de Tukey, para parámetros morfológicos de semillas de candelilla.

Localities	Weight (g)	Lenght (mm)	Width (mm)	Thickness (mm)
Lomas de Icamole	0.34 ± 0.04b	2.82 ± 0.18a	1.80 ± 0.14a	1.48 ± 0.41b
Las Marías	0.20 ± 0.06a	2.83 ± 0.23a	1.82 ± 0.19b	1.25 ± 0.38a
Las Morenas	0.42 ± 0.10c	3.05 ± 0.26b	2.12 ± 0.17d	1.76 ± 0.22c
Santa Teresa de Sofía	0.43 ± 0.07c	3.07 ± 0.23c	2.03 ± 2.12c	1.79 ± 0.21d
Total Mean	0.32 ± 0.12	2.92 ± 0.26	1.91 ± 0.22	1.50 ± 0.41

Different letters within a column indicate significant differences between localities ($p \leq 0.01$).

± 0.06 g) was recorded in seeds from Las Marías (Table 1). The ANOVA showed highly significant differences in weights among localities (p≤0.01), although no differences (Tukey, p≥0.05) were found between seeds from Las Morenas and Santa Teresa de Sofía.

Seed length, width, and thickness. Seed length showed an overall mean of 2.92 ± 0.26 mm. The longest seeds were from the Santa Teresa de Sofía locality (3.07 mm ± 0.23), and the shortest from Lomas de Icamole (2.82 mm ± 0.18) and Las Marías (2.83 mm ± 0.23). This variation among localities was statistically significant (p≤0.01). The only exceptions were Lomas de Icamole and Las Marías, which seed length did not differ significantly (p≥0.05; Table 1).

Seed widths also differed (p≤0.01) among localities with an overall mean of 1.91 ± 0.22 mm, a maximum mean of 2.12 ± 0.17 mm for Las Morenas, and a minimum mean for Lomas de Icamole (1.80 ± 0.14 mm). There was no similarity (Tukey, p≤0.05) in seed width among localities.

Seed thickness showed an overall mean of 1.50 ± 0.41 mm, with Lomas de Icamole and Santa Teresa de Sofía showing the lowest (1.48 ± 0.41 mm) and highest (1.79 ± 0.21 mm) mean values, respectively. The ANOVA showed significant differences (p≤0.01) in the thicknesses among localities (Table 1).

Viability. Significant differences were observed among the groups of seeds (p≤0.01) and among the localities (p≤0.05). In Table 2, it can be observed that seed group A (brown) showed the highest percentage of viability (86.0 ± 16.4%), whereas the amber and gray seeds had only 52.0 ± 9.77% and 21.5 ± 7.46% viability, respectively. Seeds from Santa Teresa de Sofía and Lomas de Icamole showed the highest percentages of viability for the three groups and were not significantly different (Tukey, p≤0.05). Las Morenas and Las Marías showed the lowest percentages of viability and were not significantly different (Tukey, p≤0.05).2EE

Germination under laboratory conditions. To evaluate the germination rates, only the brown seeds were considered because this group presented the highest viability rate. The germination pattern in all treatments showed that this process started almost immediately, as germinated seeds were found after the second day of the experiment. It was also observed that chemical and mechanical scarification resulted in a faster germination of seeds. Likewise, a temperature of 28 °C showed the greatest germination rate in all treatments (Fig. 1).

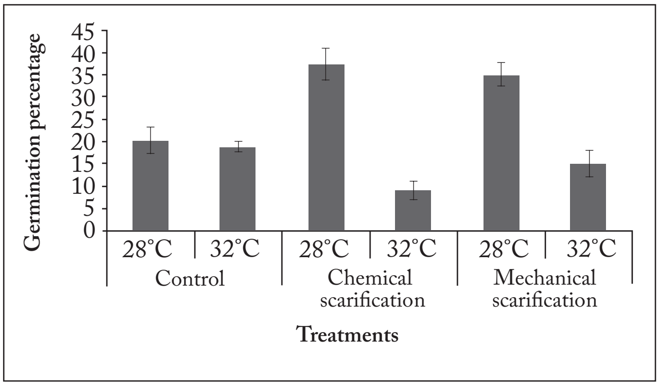

Fig. 1. Mean germination percentage of candelilla seeds with scarification treatments at 28 °C and 32 °C.

Fig. 1. Porcentajes de germinaciòn promedio de semillas escarificadas de candelilla a 28 y 32 °C.

Percentage of germination. With respect to the effects of treatments and temperature on the overall germination rate, we observed that in the control treatment, the highest rates were found at 28 °C with a variation among localities from 5 to 38%. In contrast, the total germination rates at 32 °C varied between 5 and 18% (Figs. 2A and 2B).

Chemical scarification had a positive effect on the germination rate, particularly at 28 °C. At this temperature, it reached a maximum of 60%. However, germination percentages varied between 5 and 25% among localities at 32 °C (Figs. 2C and 2D).

Mechanical scarification also produced an increase in the germination rate relative to the control, showing a mean value between 16 and 46% at 28 °C, and between 15 and 34% at 32 °C (Figs. 2E and 2F).

Table 2. Mean values ± standard deviation of candelilla seeds viability test results of the groups A, B and C.

Tabla 2. Valores promedio ± desviación estándar de resultados de la prueba de viabilidad de semillas de candelilla para los grupos A, B y C.

Localities	Group A	Group B	Group C
Lomas de Icamole	88.6 ± 10.0b	53.1 ± 9.8b	30.8 ± 6.9b
Las Marías	77.6 ± 24.7a	46.8 ± 12.4a	17.8 ± 5.8a
Las Morenas	86.0 ± 13.0a	44.6 ± 8.7a	14.7 ± 9.4a
Santa Teresa de Sofía	92.0 ± 10.3b	63.5 ± 8.2b	22.9 ± 7.7b
TOTAL MEAN	86.0 ± 16.4c	52.0 ± 9.77b	21.5 ± 7.46a

Different letters in the same column indicate significant differences between localities (p≤0.05).

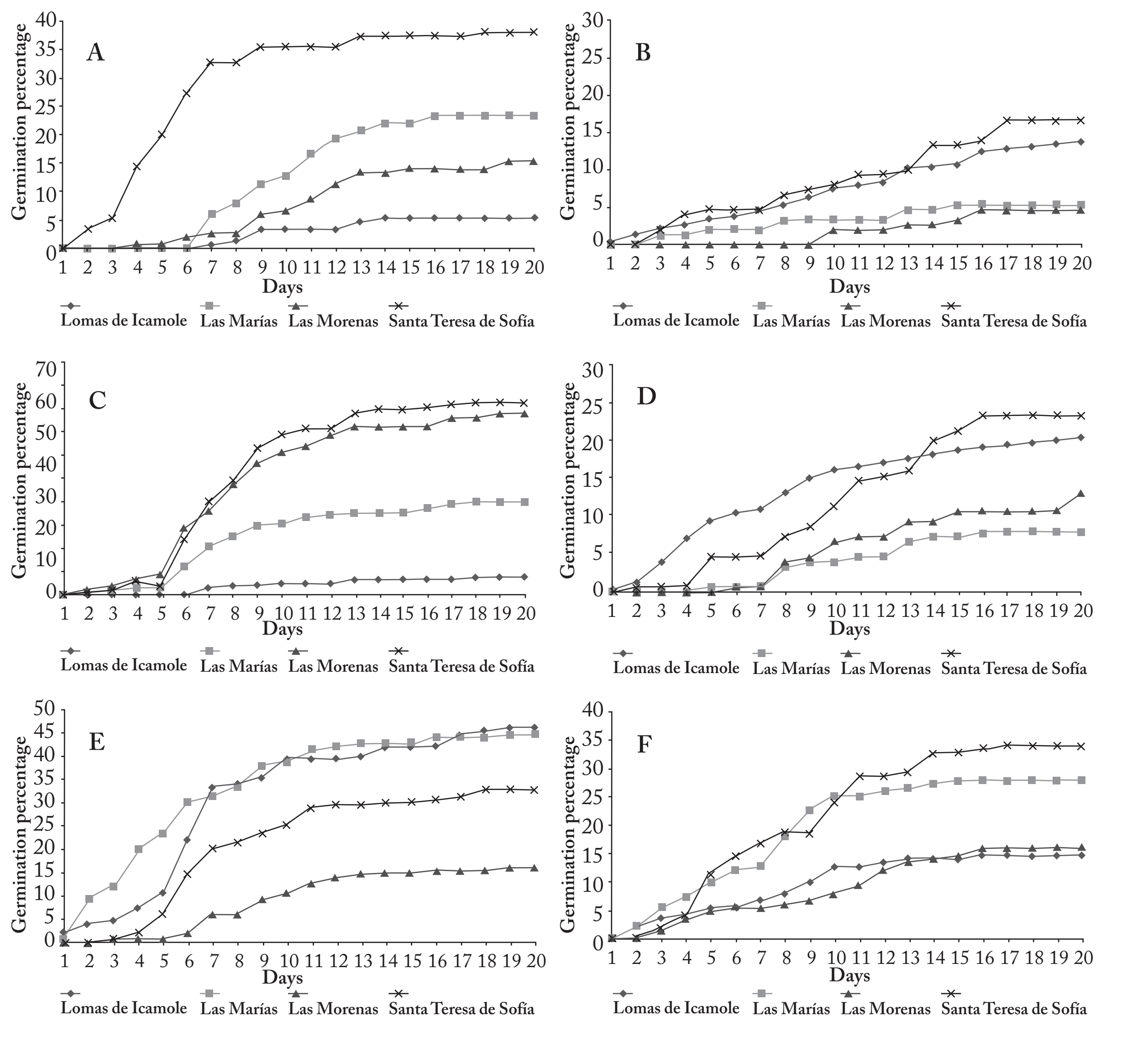

Fig. 2. Cumulative germination curve (%) of candelilla seeds from four localities in northeastern Mexico at two temperatures. Treatment 1 (control) 28 °C (A) and 32 °C (B); Treatment 2 (chemical scarification) 28 °C (C) and 32 °C (D); Treatment 3 (mechanical scarification) 28 °C (E) and 32 °C (F).

Fig. 2. Germinación acumulada (%) de semillas de candelilla de cuatro localidades en el noreste de Mexico a dos temperaturas. Tratamiento 1 (control) 28 ºC (A) y 32 ºC (B): Tratamiento 2 (escarificación química) 28 ºC (C) y 32 ºC (D); Tratamiento 3 (escarificación mecánica) 28 ºC (E) y 32 ºC (F).

With respect to the effects of seed origin on the germination rate, seeds from Santa Teresa de Sofía showed the highest germination rates for most treatments, including the control. The only exception was mechanical scarification at 28 ˚C, which was highest in seeds from Lomas de Icamole and Las Marías (Fig. 3).

Relationship between germination rate and seed weight and length. The relationship between seed weight and germination percentage was not significant (r = 0.17). The model that best fit these two variables was a linear model with a coefficient of determination of 10.2%, which indicates a weak relationship between these variables.

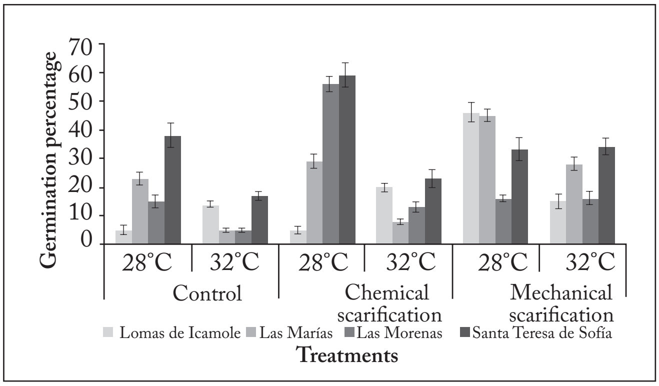

Fig. 3. Mean germination percentage of candelilla seeds from four localities in northeastern Mexico with scarification treatments at temperatures of 28 °C and 32 °C.

Fig. 3. Porcentajes de germinación promedio de semillas de candelilla de cuatro localidades en el noreste de Mexico con tratamientos de escarificación de 28 ºC y 32 ºC.

Likewise, the relationship between germination percentage and seed length was not significant (r = 0.13), and the best linear model that explained this relationship showed a coefficient of determination of 10.2%.

DISCUSSION

Esquivel (1979) reported that the shape of the candelilla seed can vary from elliptical to ovoid, and that the surface was rough. In general, this agrees with our results where most seeds showed a pyriform shape and a foveolate surface. However, this description is not in agreement with the round seeds observed in Las Morenas, or the smooth surfaces found in Lomas de Icamole and Las Marías. We found a marked predominance of brown seeds (82%), results which agree with those of Esquivel (1979), García (1988), Peña-Contreras (1998), and SEMARNAT (2005).

The mean dimensions of the seeds in this study were 2.95 mm for length, 1.95 mm for width, and 1.58 mm for thickness; these results differ from the data reported by Esquivel (1979). He stated that Candelilla seeds were 1 to 2 mm long. This discrepancy might be attributed to the precision with which the seeds were measured, the source of germplasm, and the number of replicates. A significant variation in the mean weight of 100 seeds per locality was noted, with extreme values of 0.43 g in Santa Teresa de Sofía and only 0.20 g in Las Marías. This variation had not been previously reported; however, it might have been caused by climatic variations between the localities, different genotypes, and fruit ripeness at collection.

The observed variability in the brown seeds was higher than that measured for the amber or gray seeds in all localities, with mean values of 77.6 to 92%. This variability was directly related to seed maturity because only the brown seeds reached the appropriate maturity. The germination pattern observed in the present study showed that the pre-germination treatments (i.e., chemical and mechanical scarification) resulted in a faster germination than the control. This effect was most likely due to the thinning of the seed coat caused by the pre-germination treatments, which favored seed imbibition. The chemical and mechanical scarification of candelilla seeds yielded higher mean values for the germination rates relative to the control, with values of up to 60% for chemical scarification at 28 °C, and up to 38% at 32 °C for the control. This observation differs from the report of Villa-Castorena et al. (2005), who showed that candelilla seeds without a pre-germination treatment had germination rates of 62%. However, their work was performed under greenhouse conditions, at room temperature, and by planting the seeds in plastic bags, germination trays, and polyurethane cups using peat moss with perlite, and peat moss with sand as substrate.

Finally, it is important to note that the enhanced efforts for the conservation of candelilla populations (Dávila, 1981; Tovar-Villa et al., 1992; Villa-Castorena et al., 2005; CONAFOR, 2008) have not sufficed. Therefore, it is necessary to further investigate strategies for the propagation of candelilla which include sexual or asexual propagation, a greater diversity of germplasm sources, various conditions of propagation, and techniques for the re-introduction of candelilla in the field. This will contribute to define appropriate strategies for the reforestation of the large areas where the populations of this species have decreased or disappeared. We have to consider as well the development of management techniques and strategies that allow for the sustainable use of this important plant resource.

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