Solvent pre-treatment effect on germination of creeping devil cactus (*Stenocereus eruca)* seeds

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ABSTRACT

Stenocereus eruca is a columnar horizontally grown cactus that can "move" on the ground relatively fast. It is considered an endangered species and endemic to a very small extension of land in Baja California Sur, México. Seedling recruitment seems to be a big problem for its propagation; however, it can propagate asexually. There is strong evidence that seed pre-treatment with organic solvents affect seed germination ability. Thus, the objective of this study was to determine the effect of treating chirinola seeds, collected from Puerto San Carlos area, with different solvents in terms of rate and total germination. The experiment was arranged in a completely randomized design using two different solvents (acetone and dichloromethane) with four replicates of 25 seeds each one. Pre-treatment of chirinola seeds with acetone increased total germination almost double (62%) as compared to the control (32%). On the other hand, pre-treatment of the seeds with dichloromethane reduced total germination to 0%. The use of this two solvent for pre-treatment of the seeds had very different effect of germination making acetone a suitable solvent in order to increase total germination of chirinola seeds.

Keywords: Chirinola, cactus endangered species, germination, solvent treatment.

INTRODUCTION

Stenocereus eruca (Brandegee) Gibson & Horak is known as creeping devil cactus and, in Mexico, as "chirinola". It is an endangered species according to Mexican legislation (NOM-059-ECOL-2001), mainly threatened by destruction of its habitat due to agricultural development, tourism development and illegal collection (Cancino *et al.* 1995; Clarck-Tapia *et al.* 2005a). This species is endemic to a very small area of Baja California Sur, Mexico near Magdalena Bay (Fig.1). *Stenocereus eruca* shares many botanical features with *Stenocereus gummosus*; actually, many years ago, only these two species belonged to the genus *Machaerocereus*, but after some time they were included in the bigger genus *Stenocereus* (Bashan *et al.* 2003).

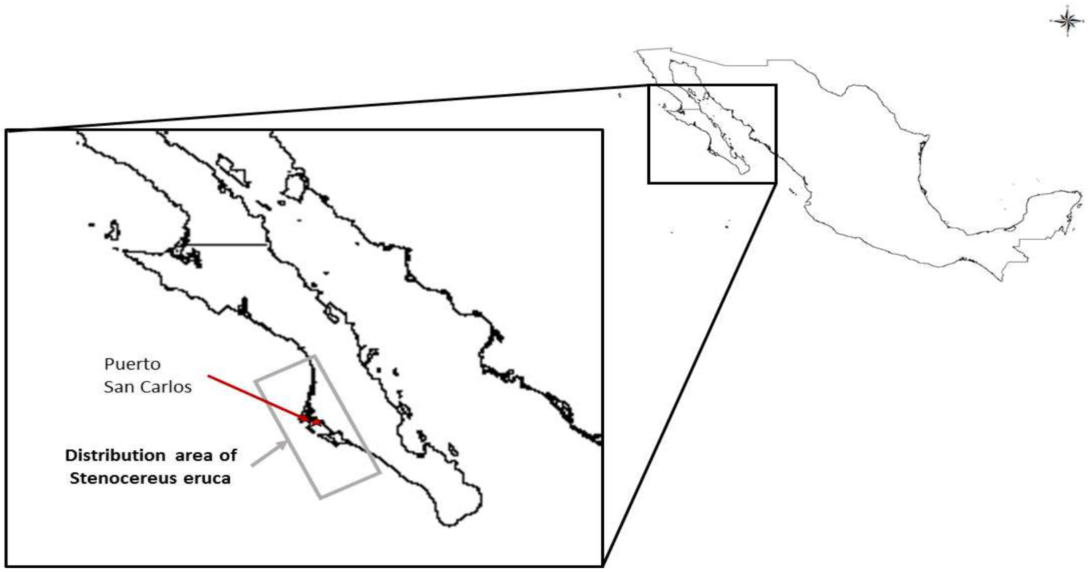

Figure 1. *Stenocereus eruca* distribution region.

This cactus does not grow erect but horizontally in such a way that continuously grows forward; new tissue is developing in the growing front and old tissue is dying at the back. This feature is possible due to its vegetative way of propagation. Some studies have shown that, in proper conditions, it can grow up to 60 cm per year and in less appropriate conditions it can only grow 6 cm per year. Despite *Stenocereus eruca*´s clonal propagation, sexual reproduction seems to be very important for regeneration according to genetic studies (Clark-Tapia *et al.* 2005a); however, no seedling recruitments have been observed in *Stenocereus eruca´s* wild populations. Perhaps, new seedlings might be established during rare episodes; for example, during flooding events (Clark-Tapia *et al.* 2004; Clark-Tapia *et al.* 2005b).

Creeping devil cactus grows in open areas and the vegetation around the plant is primarily small shrubs like *Euphorbia californica* and other *cactaceae* like *Stenocereus gummosus* and *Opuntia* spp. (Figure 2) (Bashan *et al.* 2003).

As an effort to increase *Stenocereus eruca* seedling production, seed pretreatment can be used as a strategy to promote seed germination. There are many types of seed pretreatments like hydropriming, physical (scratch) and chemical (acids) coat scarification applied to *Cactaceae* seeds (Mandujano *et al.*, 2005).

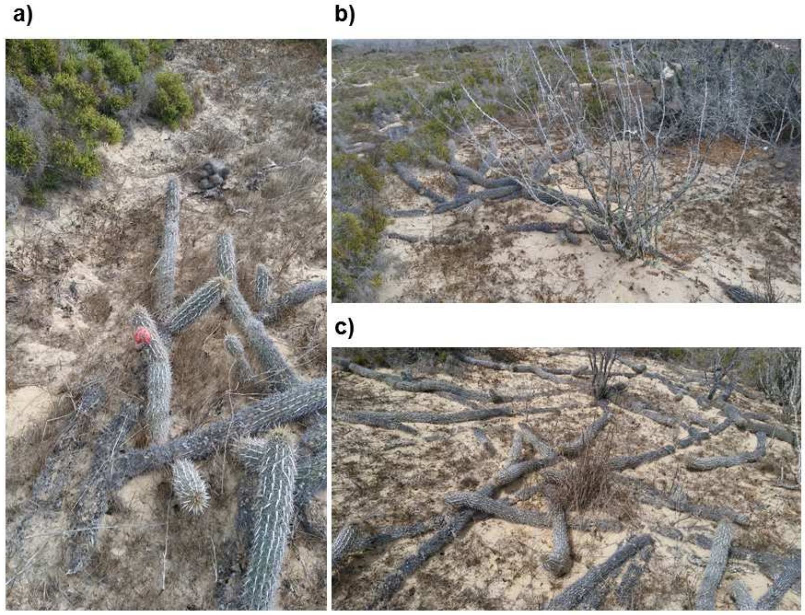

Figure 2. a) Chirinola adult plant with fruit. a), b) and c) chirinola adult plants association with surrounding vegetation. Credit: Pablo Misael Arce-Amezquita

Pretreatment of seeds with organic solvents affect their germination (Subbaiah, 1982). In some cases, the solvent promotes seed germination and in others, it is deleterious to the seed. In addition, organic solvents have been used for infusing chemicals like hormones and antimicrobial compounds into dry seeds without affecting seed germination (Tao *et al.* 1974; O'Neill *et al.* 1979). Therefore, the objective of this study was to determine the effect of treating chirinola seeds, with different solvents that have been shown to be compatible in terms of germination rate and total germination. To our knowledge, this is the first time that an organic solvent has been used for increasing total germination of cacti seeds.

MATERIALS AND METHODS

Area of study

Stenocereus eruca ripe fruits (red color) were collected in November 2015 nearby Puerto San Carlos, Baja California Sur, Mexico (24°48´48´´ North Latitude, 112°5´13´´ West Longitude). Puerto San Carlos is a small town located at the pacific coast of Baja California Sur and it is part of the Magdalena plains area (Fig. 1). Because *Stenocereus eruca*´s distribution area is on the pacific side of the Peninsula, it has very different weather. The minimum and maximum average temperatures do not vary too much for most of the year; only in two months (August and September) average temperatures just pass 30°C (Fig. 3). This condition is very different if compared to most of the areas of the state, which can easily reach far higher temperatures (>40°C for prolonged periods).

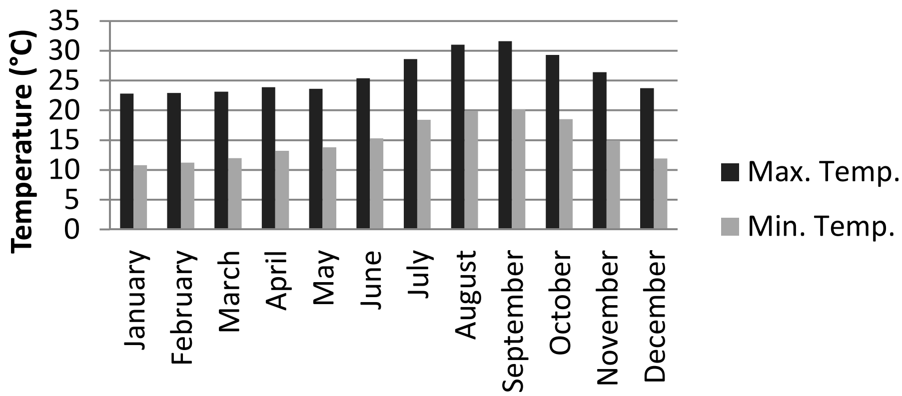

Figure 3. Puerto San Carlos monthly maximum and minimum average temperatures (1961-2003) (Ruiz-Corral *et al.* 2006).

Puerto San Carlos area, as part of arid region, has occasional rain events (average total annual precipitation 98.4 mm from 1961 to 2003) and high rates of evaporation (Ruiz-Corral *et al.* 2006). Primarily, rain happens during autumn and winter. Precipitation during the hottest months of the year is mainly due to the incidence of atmospheric phenomena like hurricanes or tropical storms. Interestingly, climatological data shows much more evaporation than precipitation in any month of the year, at least four times more (Figure 4). The extra humidity probably comes from the Pacific mist.

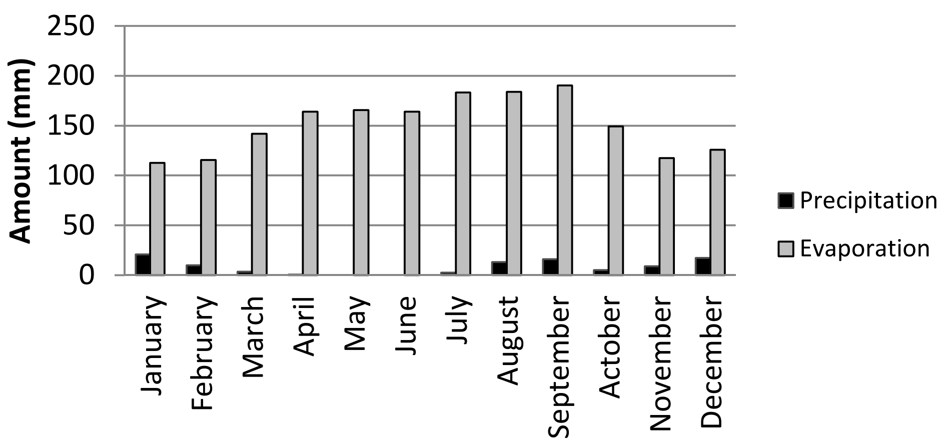

Figure 4. Puerto San Carlos monthly average precipitation and evaporation (1961-2003) (Ruiz-Corral *et al.* 2006).

Seed preparation

The fruits were dissected and sun dried; then, the seeds were separated, washed, dried and kept at 4°C (for one year) in sealed vials before germination test. For germination test the seeds were pre-treated, or not, by soaking them for 8 h in acetone or dichloromethane, using

30 mL of solvent per gram of material. Seeds were filtered and dried in an oven at 40°C for 30 min under diminished pressure before sowing.

Germination conditions

Twenty five seeds were placed on four layers of filter paper (Whatman™ No.1) in 9 cm-diameter plastic Petri dishes containing 10 mL of distilled water. The dishes were sealed with parafilm and kept at 23°C for 14 days under continuous cool white fluorescent light (3.12 μmol m^{-2} s^{-1}). The seed coat rupture was considered as indication of germination (Figure 5).

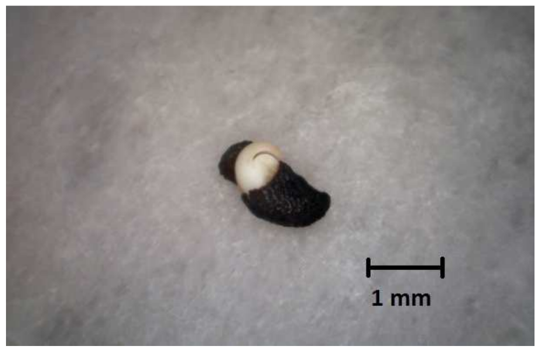

Figure 5. Chirinola germinated seed. Credit: Felix Alfredo Beltrán-Morales.

Germination was recorded once a day during the length of the experiment and total germination (TG), mean germination time (MGT) were calculated by means of:

$$\mathrm{TG} = \frac{n}{N} \times 100 \qquad \mathrm{MGT} = \frac{\sum(n \times t)}{\sum n}$$

Were *t* is time in days, *N* is the total number of seeds sown and *n* is the number of germinated seeds on day *t*. Germination rate index (GRI) was calculated using Maguire equation (Maguire, 1962):

$$\mathrm{GRI} = \sum \frac{n}{t}$$

Were *t* is time in days and *n* is the number of germinated seeds on day *t*.

Statistical analysis

The experiment was arranged in a random design that consisted of four replicates of 25 seeds per solvent treatment (acetone and dichloromethane) and four replicates of 25 seeds for the control. Data were examined using one-way univariate analysis of variance (ANOVA), type of solvent for seed treatment was used as a fixed factor (no solvent, acetone and dichloromethane). The difference between the means was determined by Tukey´s test HSD multiple range test at p=0.05. Mean values were considered significantly different when $p \leq 0.05$. Statistical analysis was carried out using Statistica™ v. 10.0.

RESULTS AND DISCUSSION

Immersion of *Stenocereus eruca* seeds in two solvents caused significantly different responses (p=0.05). Seed pre-treatment with acetone increased total germination, around double, as compared to the control. The control showed 33% total germination in accordance to what is reported in literature (Yang *et al.* 2003; Clark-Tapia *et al.* 2005b). However, pre-treatment with acetone increased the total germination to 62%. On the other hand, seed pre-treatment with dichloromethane eliminated germination (Figure 6). As an important fact, after day 9 only very few seeds germinated for acetone treatment and control. According to MGT and GRI this means that more seeds germinated for acetone treatment than control at very similar rates.

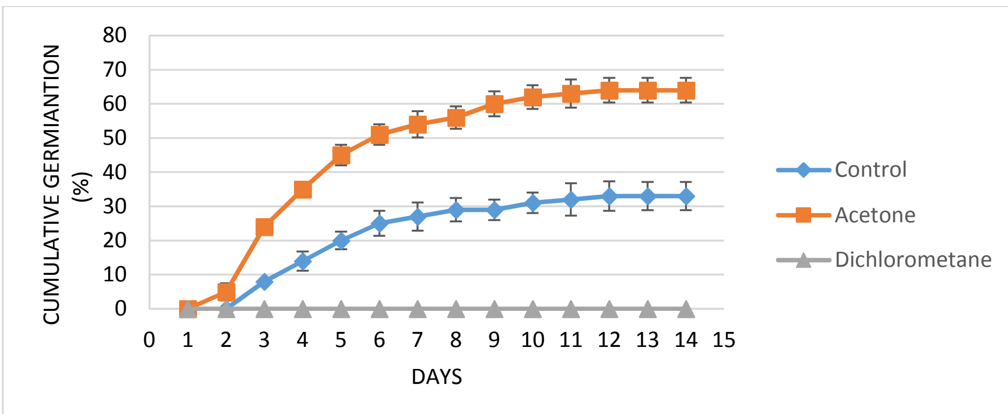

Figure 6. Effect of solvent pre-treatment of seed on percentage of total germination. Values are means ± S.E.

Increase in total germination due to pre-treatment with acetone has been observed in other seeds species like lettuce (Rao *et al.* 1976), corn (Hung *et al.* 1992), cashew (Subbaiah, 1982) and pepper (Sachs *et al.* 1980). Actually, acetone is a compound that forms naturally in seeds during lipid metabolism (Murphy, 1985); therefore, acetone is a biocompatible solvent for seed treatment. On the contrary, dichloromethane ended up abolishing any possible germination most likely due to toxicity to the embryo. This solvent was used because it has been shown to be compatible for lettuce and soybean seeds germination (Khan *et al.* 1973; Shortt *et al.* 1980).

The solvents used for this experiment have completely different chemical properties; acetone, for instance, is more polar solvent (dielectric constant = 21.0) than dichloromethane (dielectric constant = 9.08). Also, dichloromethane is a halogenated solvent. This property makes acetone a miscible solvent and dichloromethane immiscible with water. Such properties might play a determinant role in the process of germination of *Stenocereus eruca* since soaking the seeds in the solvents used herein may affect the level or structure of molecules within the seeds (i.e. promoters or inhibitors of germination) or may as well compromise seed coat integrity. In an extreme case, the solvent might be toxic for the embryo affecting negatively the processes of germination. Clearly, acetone could be removing those molecules that inhibit germination from the seed tissues, it could be activating molecules that promote germination or it could be weakening the seed coat, without affecting mean germination time but increasing significantly the total germination and germination rate as compared to the control. On the other hand, dichloromethane could be removing molecules that promote germination or important molecules like fats which are an important source of energy during this stage of development (Table 1).

Table 1: Effect of acetone and dichloromethane pre-treatment on total germination (TG), mean germination time (MGT) and germination rate index (GRI) of *Stenocereus eruca* seeds.

	TG (%)	*MGT (days)*	*GRI*
Control	33 ± 4.1 a	9.4 ± 0.12 a	9.67 ± 1.0 a
Acetone	62 ± 3.5 b	9.2 ± 0.10 a	21.2 ± 1.1 b
Dichloromethane	0 c	0 b	0 c

Values are mean ± S.E. Values within the same column with the same letter are not significantly different at p=0.05 (Tukey´s test HSD multiple range test).

New chirinola seedlings obtained outdoors or in greenhouse have low percentage of survival after one year (average 0.002) (Clark-Tapia *et al.* 2005b). Therefore, it is of interest, in further experiments, to study the survival capacity of seedlings obtained from seeds pre-treated with acetone. Also, the effect of other scarification methods on germination of *Stenocereus eruca* seeds is of interest, especially because we did not find any information on this topic.

Ethics statement

The research conducted herein did not involve experiments with humans or animals. The study site is not considered a protected area; however, an endangered species was used for this study. Permission needed for using this material was granted by Saúl Colín Ortiz, a Federal Officer of PROFEPA (Procuraduría Federal de Protección al Ambiente) in Baja California Sur. For future collections of *Stenocereus eruca* and other endangered species in the study area, appropriate permissions of PROFEPA will be requested.

CONCLUSIONS

It was explored the possibility of using organic solvents for improving total germination in cacti seeds, ergo, increasing the total germination. The effect of soaking the seeds in acetone successfully increased the germination of *Stenocereus eruca*. The possibility of using other solvents needs to be explored as well as the length of the seed treatment. Also, this treatment has the potential to be used with other cactus species, in order to increase total germination, especially for those that have very low percentages of propagation, like the one studied in this paper. Furthermore, seed pretreatment with organic solvents could be used, in future experiments, for infusing other substances, like antioxidants, into the seeds in order to enhance seedling vigor thus increasing production of *Stenocereus eruca* seedlings for conservation purposes.

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