Effect of imbibition treatments on the germination of *Stenocereus zopilotensis* (Cactaceae) native from Guerrero, Mexico

Efecto de tratamientos de imbibición sobre la germinación de semillas de *Stenocereus zopilotensis* (Cactaceae) nativas de Guerrero, México

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SUMMARY

The prickly pear cactus "tuna pelona" (*Stenocereus zopilotensis*), is an endemic cactus species from the state of Guerrero, Mexico, it develops in semi-arid areas and has great ecological importance as it houses and forms part of the diet of multiple species in its niche. There is a constant threat to natural populations of this species due to the overexploitation of seedling collections in the field by unregulated trade. In order to design an alternative for the conservation management of these species, the objective of the work was to evaluate treatments for the imbibition of "hairless tuna" seeds to promote germination and the successful establishment of seedlings with economic technologies for the farmers of the area. Fruits of "tuna pelona" (*S. zopilotensis*) were collected, near the community of Xalitla, municipality of Tepecoacuilco de Trujano, Guerrero; the seeds were stored for 70 and 273 days after collection (dac) at room temperature in order to maintain adequate conditions for the conservation of viability until reaching dormancy. In the stored seeds, the effect of soaking treatments was evaluated: 0, 6, 12, 24 and 12 hours in water plus another treatment that consisted of immersion in a plant-based hormonal stimulant. The germination percentage per treatment was evaluated, it was found that the control treatment (zero hours in water) of seeds stored at 70 dac only obtained 2.4% of germination. The treatment of soaking in water and in the hormonal stimulant both for 6 h presented the highest germination values with 48.6 and 44.4% respectively when using seeds of 273 dac at 14 days after the application of the treatments. These results will allow the massive production of seedlings of the species for reforestation and regional marketing purposes.

Index words: *dormancy, latency, soaking, stimulation.*

RESUMEN

La "tuna pelona" (*Stenocereus zopilotensis*), es una especie cactácea endémica del estado de Guerrero, México, se desarrolla en zonas semiáridas y tiene una gran importancia ecológica al albergar y formar parte de la dieta alimenticia de múltiples especies de su nicho. Existe una amenaza constante de poblaciones naturales de esta especie debido a la sobreexplotación de colectas de plántulas en campo por comercio no regulado. Con el fin de diseñar una alternativa para el manejo conservacionista de estas especies, el objetivo del trabajo fue evaluar tratamientos de imbibición de semillas de "tuna pelona" para promover la germinación y el establecimiento exitoso de plántulas con tecnologías económicas para los campesinos del área. Se colectaron frutos de "tuna pelona" (*S. zopilotensis*), cerca de la comunidad de Xalitla, municipio de Tepecoacuilco de Trujano, Guerrero; se

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almacenaron las semillas por 70 y 273 días después de la colecta (ddc) a temperatura ambiente con el objetivo de mantener condiciones adecuadas para la conservación de viabilidad hasta alcanzar la latencia. En las semillas almacenadas se evaluó el efecto de tratamientos de remojo: 0, 6, 12, 24 y 12 h en agua más otro tratamiento que consistió en inmersión en un estimulante hormonal de origen vegetal. Se evaluó el porcentaje de germinación por tratamiento, se encontró que en el tratamiento control (cero horas en agua) de semillas almacenadas a los 70 ddc, solo se obtuvo 2.4% de germinación. El tratamiento de remojo en agua y en el estimulante hormonal ambas por 6 h presentaron los valores más altos de germinación con 48.6 y 44.4% respectivamente al utilizar semillas de 273 ddc a los 14 días después de la aplicación de los tratamientos. Estos resultados permitirán la producción masiva de plántulas de la especie para fines de reforestación y comercialización regional.

Palabras clave: *dormancia, estimulación, latencia, remojo.*

INTRODUCTION

Mexico is the most important center of the world of cacti concentration. Cactologists recognize the existence of 913 taxa, making up 669 species, which are grouped into 63 genera (Guzmán *et al*., 2003). By geographic region, it can be found the greatest diversity in the Tehuacán – Cuicatlán valley (Puebla and Oaxaca), followed by the Barranca de Metztitlán and the Balsas depression (Guzmán *et al*., 2003). The "cañada del zopilote" is an area located within Balsas river depression and is recognized as a physiographic area with a high concentration of taxa, among them is *S. zopilotensis* (Arreola-Nava and Terrazas, 2004). In this region, cacti have great cultural, nutritional and ecological importance, being the sustenance and shelter of great diversity of other plants and animal species (Alanís and Velazco, 2008). The natural populations of many species have been affected by the pressures of human development, mainly due to the conversion of natural areas for agricultural and/or livestock uses, also the plants extraction from their habitat for sale as decoration plants in national and international markets affect their populations, so it is important to implement effective propagation technologies to minimize the impact on wild species (Jarvis, 1979; Sánchez-Mejorada, 1982; Fuller and Fitzgerald, 1987).

Most plant seeds are exposed to very extreme seasonal periods of time during which they can be damaged or die if they do not have some protection mechanism. Dormancy is the most widely used mechanism in many seeds from cacti species, and is commonly induced by unfavorable conditions (cold or extreme dry heat). This phenomenon can be defined as a state of suspended or slowed growth and metabolism. Lethargy is a survival mechanism against frost, drought, etc. and it is necessary for populations of many plant species; it must occur in due time (that is, before adverse conditions reach lethal intensity) and last long enough, it must be interrupted when conditions are ideal for growth and development to resume (Matilla, 2008). The main mechanisms that cause lethargy in the seed are: 1. Environmental factors such as light, temperatures and absence of water; 2. Internal factors: such as test characteristics, embryo immaturity, ethylene concentration, presence of inhibitors, absence of growth promoters, and 3. Timing mechanisms: post-maturation, inhibitors blocking and synthesis of growth promoters (Benítez-Rodríguez *et al*., 2004; Benítez, 2009[1]; Flores *et al*., 2006; Sánchez-Soto, 2003[2]).

The germination process consists in water absorption, metabolism reactivation and growth initiation; most seeds begin to germinate as soon as they are wet, as long as the temperature, light and cold pretreatment conditions are optimal (Baskin and Baskin, 1998; Gutterman, 1993). It is important to know the optimal germination conditions for the efficient establishment of production units such as seedlings and nurseries. Germination and establishment are the most critical stages in life cycle of plants, because they are more vulnerable to environmental stress, competition, predation and diseases (Angevine and Chabot, 1979; Fenner, 1985). Little information was found on the optimal germination conditions of *S. zopilotensis*; many cacti need specific conditions to carry many of these physiological processes. It has been reported that in some *Stenocereus* species,

[1] Benítez G., D. G. 2009. Germinación de semilla de *S. zopilotensis* Arreola-Nava H. J y T. Terrazas, de la cañada de Zopilote, Guerrero. Tesis de Licenciatura. Unidad Académica de Ciencias Agropecuarias y Ambientales, Universidad Autónoma de Guerrero. Iguala, Guerrero, México.

[2] Sánchez-Soto, B. H. 2003. Germinación de Semillas de Cinco Especies de Cactáceas del Desierto Costero de Topolobampo, Ahome, Sinaloa. Tesis. Colegio de Postgraduados. Montecillo, México. 90 pp.

that the seeds increase their germination percentage with the storage age both in constant and fluctuating temperatures (Rojas-Aréchiga *et al*., 2001). The effect of previous soaking in seeds of different cacti has been reported as a treatment can increase the germination percentage (De Carvalho *et al*., 2008; Gonzalez-Cortés *et al*., 2018). Based on the above, the objective of this study was to evaluate treatments of immersion in water and hormonal solutions of *S. zopilotensis* seeds on the break of dormancy and germination.

MATERIALS AND METHODS

Description collecting area. 150 fruits were collected from five *S. zopilotensis* plants near the community of Xalitla, municipality of Tepecoacuilco de Trujano, Guerrero. The research material collection area was located on slopes with slight slopes, due to hills located to the east, with regularly flat areas that have been used for agriculture; The area is located at an altitude of 740 meters above sea level. In the western part is the Zopilote River, which receives the Coloapan and Huacapan rivers currents, which descend from the south-eastern region and flow only in the rainy season (INEGI, 2017). Based on the Köppen classification, the climate is warm semi-humid with summer rainfall; the annual mean temperature was 29.7 °C and precipitation was 586.16 mm (CFE, 2017[3]). In the study area, the vegetation type is low deciduous forest, in which predominate species are: *Ceiba aesculifolia* "ceiba", *Cyrtocarpa procera* "berraco", around 20 Burseras species, as *B. morelensis*, *B. xochipalensis*, *B. lancifolia*, *B. longipes* among others (Ávila *et al*., 2010); *Zizyphus acuminata* "corongoro", *Lysiloma tergeminum* "goat leg", *Euphorbia schlechtendalii* "milkweed", *Neoevancia zopilotensis* and *Neobuxbaumia* sp. "cactus mezcala ".

Seed drying and storage. Physiological maturity fruits of *S. zopilotensis* "tuna pelona" were collected, were taken to the plant physiology laboratory of the Academic Unit of Agricultural and Livestock and Environmental Sciences in the University of Guerrero in Iguala, Guerrero, Mexico; where the seeds were extracted (5000 for all the study). The seeds were washed under running water and dried in the shade under room temperature conditions. The seeds were stored in cool conditions (17-19 °C) to try to keep their viability.

Latency break treatments. The collected seeds (same collection) were divided into two groups based on the storage time, the first by 70 dac (days after collection) and the second by 273 dac based on other studies (Alvarez-Espino *et al*., 2014). Each group seeds were stirred and randomly separated into sets of 100. The seeds were deposited in plastic containers, in each one a set of 100 seeds was placed (repeat) where the different treatments were applied. The established treatments were: T1: no soaking; T2: 6 hours soaking in 500 mL of water; T3: 12 hours soaking in 500 mL of water; T4: 24 hours soaking in 500 mL of water, and T5: 6 hours soaking in 500 mL plant-based hormonal stimulant Biozyme TF® (gibberellins - AIA - Zeatin, 32.3 - 32.2 - 83.2 mg L^{-1} respectively), Seed Anatomy and Water Uptake in Relation to Seed Dormancy in Opuntia tomentosa (Cactaceae, Opuntioideae) (Alvarez-Espino *et al*., 2014). Five repetitions per treatment were used, giving a total of 5000 seeds, 2500 for each group (70 and 273 dac). Once the latency break treatment was finished, the seeding was carried out, for this, plastic Petri dishes 8.5 cm in diameter and 1.4 cm high were used, to which circles of paper towels (sanitas®) were placed, before placing the seeds on the paper, were moistened with running water and maintained at 20±2 °C.

Seed germination. From the seed placement, germination was recorded in all treatments every 24 h, this due to the responses observed in previous studies. for the statistical analysis, the germinated seeds were quantified and at 5, 10 and 14 days after treatments application (based on the germination period commonly found in cacti) in both groups (70 and 273 dac); during this time, moisture of Petri dishes was kept by adding running water. The experiment was replicated for each type of seed.

Experimental design and statistical analysis. The experimental design was a completely random factorial design, product of five treatments and two types of seeds (70 and 273 dac), using five repeats (100 seeds group) per treatment, giving 50 experimental units total. The experimental units where the treatments were applied were randomized using the "design.ab" procedure in the "R" statistical program, the above to ensure the independence of the observations. With the data without transforming of germinated seeds, an error normality distribution analysis was performed using the Shapiro-Wilk test and homogeneity of variances

[3] CFE (Comisión Federal de Electricidad). 2017. Boletín climatológico. Comisión Federal de Electricidad. No 17, años 2011- 2015. México, D. F.

analysis with the Bartlett test; An analysis of variances test and comparison of means was performed using the Tukey test ($P \leq 0.05$) with the statistical software SAS V.9.4 (SAS, 2013).

RESULTS AND DISCUSSION

The germinated seeds records per treatment (five repetitions sum) in both groups are shown in Table 1. During the three evaluation dates, a constant behavior was registered in the treatments with six, 12 hours soaking in water and six hours soaking in Biozyme using seeds with 273 days of storage; Although germination in the first days was minimal, it increased significantly on the second evaluation date and an equally high peak was presented on the third (Table 1).

Consistently, the dormancy breaking treatments obtained significant results regarding the germinated seeds; Furthermore, it was evident that using of seed with 273 days of storage yielded the best results (Figure 1).

Analysis of variances test of third data record showed that, with respect to "storage time" factor, the highest levels of germinated seeds were obtained with the material of 273 days of storage (Table 2). Other authors have reported different germination patterns of cactaceous seeds with respect to storage time: seeds that lose viability at year, seeds that remain viable and germinate similarly for up to two years, and seeds where germination increases with breaking dormancy at 1 to 2 years old (Flores *et al*., 2005, 2008; Flores and Jurado, 2011). Similar to that reported in the present work, Flores *et al*. (2005) observed in some cacti greater germination levels in seeds of one-year-old or more, than in newly obtained seeds; this was also correlated with seed size; Flores *et al*. (2005) concluded that higher germination levels were determined in small 4-year-old seeds of *Turbinicarpus* sp. compared to larger young seeds; this could be related to the capacity of water absorption or as a survival strategy; thus they may become almost completely dehydrated during times of drought, shutting down all metabolic processes until water becomes available again (Ogburn and Edwards, 2010). It is documented that the hydrating capacity of water generates changes in the turgor of plant cells; This turgor is directly related to the enzymatic and metabolic activity; Usually this activity presents a Gaussian behavior with respect to the turgor and the water potential of the tissue, in this case of the seed; the above could explain the behavior of *S. zopilotensis* seeds in this study, where imbibition times greater than 6 hours caused a drop in germination percentage (Bradford, 1995; Kierzkowski *et al*., 2012). Embryonic immaturity is a factor that can cause innate dormancy, so seeds need a later maturation period to germinate, and this varies for each species (Rojas-Aréchiga and Vázquez-Yanes, 2000). This has been reported in various cacti species such as *Eriocereus bonplandii* and *Mammillaria zeilmanniana*, whose seed germination rate increased with age (Zimmer, 1967, 1969); likewise, young seeds of *Ferocactus latispinus* var. spiralis germinated less than 50%, while 45 month old seeds germinated more than 80% (Zimmer, 1980). This has also been demonstrated in *Opuntia rastrera* seeds (Mandujano, 1995[4], Mandujano *et al*., 1997) and *Sclerocactus polyancistrus* where the seeds must be "aged" before germination can occur (May, 1994). For several cactus species, a period of maturation or aging

Table 1. Register of accumulate germinated seeds of *S. zopilotensis* using dormancy breaking treatments.

	Control		6 h in water		12 h in water		24 h in water		6 h in Biozyme‡	
dat†	70 dac	273 dac	70 dac	273 dac	70 dac	273 dac	70 dac	273 dac	70 dac	273 dac
5 days	0	0	8	8	6	0	0	0	0	2
10 days	7	4	18	198	12	87	17	2	3	177
14 days	12	10	30	243	15	124	25	14	11	222

† Days after treatments; dac = days after collection. ‡ Mix of Gibberellin - AIA - Zeatina, 32.3 - 32.2 - 83.2 mg L^{-1} respectively.

[4] Mandujano-Sánchez, M. C. 1995. Establecimiento por semilla y propagación vegetativa de Opuntia rastrera en dos ambientes constantes en la reserva de la biósfera de Mapimí, Durango. Tesis de Doctorado. Centro de Ecología, UNAM, México, D. F.

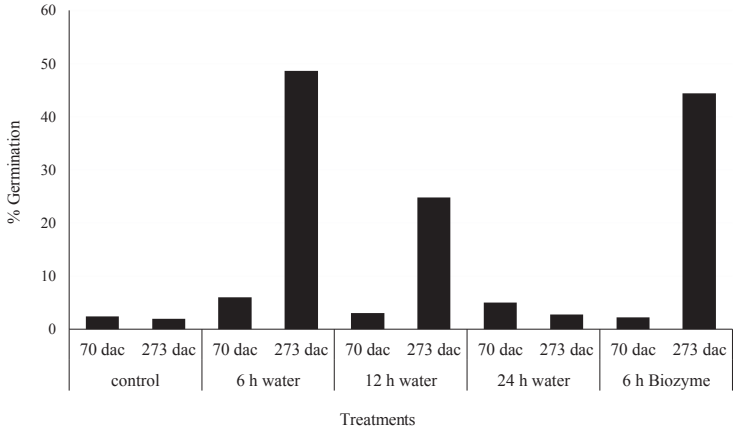

Figure 1. Seeds germination of *S. zopilotensis* through the interaction of dormancy breaking treatments and storage at 14 days after treatments application.

after harvest is necessary to occur germination or to be high (Rojas-Aréchiga and Vázquez-Yanes, 2000); Also, these authors mentioned that the Information concerning the dormancy and germination behaviour of cactus seeds comes from the pioneering studies of Alcorn and Kurtz (1959) and McDonough (1964), which demonstrated that light has a stimulating effect on the germination of *Carnegiea gigantea* and *Stenocereus thurberi*.

The statistical analysis determined that, with respect to the factor "latency breaking treatment", the best option to induce the germination starting process was T2: 6 hours soaking in water and T5: 6 hours soaking in hormonal stimulant Biozyme TF®, regardless of seeds storage time (Table 3). This indicates that in the presence of water, hydration sensitizes the seed tissues, and they respond by activating metabolic activity which starts the germination process, coinciding with what was proposed and previously mentioned by Ogburn and Edwards (2010); beyond 6 hours of soaking in water, the metabolic activity decreases, modifying this process. It is important to mention that, although the soaking treatment in running water for 6 hours was the most successful, a good part of the seeds did not germinate, so it is necessary to look for more alternatives that significantly increase the germination percentage (Matilla, 2008). A seed with 5-10% water content has a very negative water potential, so it tends to imbibrate very quickly, this rapid phase of water absorption causes temporary alterations in the seed membranes differential permeability, and consequently, a solutes loss to the surrounding environment; also lossing of different low molecular weight metabolites such as sugars, organic acids, ions, amino acids, germination inhibiting peptides (such as phenols) and ABA (Matilla, 2008). After the initial imbibition phase,

Table 2. Means multiple comparison of germination percentage of *S. Zopilotensis* seeds of 70 and 273 dac in the first trial.

Group (dac)	Mean
273	24.52 a*
70	3.76 b

dac = days after collection. * Means with different letters in the same column differ statistically according to the Tukey test ($P < 0.05$).

Table 3. Means multiple comparison of the germination percentage of *S. Zopilotensis* seeds under latency breaking treatments.

Treatments	Mean
T 6 h	27.60 a*
T Bio	23.30 ab
T 12 h	13.90 bc
T 24 h	3.70 dc
T 0 h	2.20 d

* Means with different letters in the same column differ statistically according to the Tukey test ($P < 0.05$).

the membranes regain their more stable configuration and the loss of solutes is reduced, a few moments after the imbibition of the viable seed begins, their metabolic activity resumes (Matilla, 2008).

Similar results to ours were reported by Sánchez-Salas *et al*. (2006), who found that in *Astrophytum myriostigma* the best treatments for germination were distilled water and cooling, while the treatment with the lowest percentage of germination was mechanical scarification. Similar results were reported by Navarro and Deméneghi (2007); who applied dormancy break treatments in *M. pectinifera*, they found that the seeds germination percentages varied between the different treatments, the highest percentage (95%) was with the Control (water) treatment, the gibberellin and sulfuric acid treatments registered similar values 85 and 80% respectively, while the lowest value (50%) was obtained when seeds immersed in Tween®. Similarly, Villanueva *et al*. (2016) reported that when applying dormancy breaking treatments in *E. platyacanthus* and *M. pectinifera* seeds, the highest average germination rates and germination speed were observed in water immersion treatments, this was previously reported by Mihalte *et al*., 2011. this suggests hydration sensitizes the seeds so that they can respond quickly to other environmental factors that trigger germination processes, such as enzymatic activation, synthesis of ABA inhibitors, as well as during the phase of seeds imbibition with PDND (physiological dormancy not deep) the several genes induction that respond to GAs (giberellins), phytohormones that lead to the embryo development and the subsequent emergence of the radicle through the testa takes place (Matilla, 2008). In addition to the above, the factorial design allowed analyzing the combinations between storage time and latency breaking treatments, ANOVA confirmed that the best combination to induce germination in "tuna pelona" was using seeds of 273 days of storage and apply treatments: soaking in water (6 hours) or soaking in Biozyme TF® hormonal stimulant (6 hours) and the second best option was soaking in water for 12 hours, these results could indicate the imbibition time as a determining factor, however, it is advisable to extend the study by adding combinations of hormonal solutions and soaking times to determine the effects of these variables. (Table 4).

Table 4. Means multiple comparison of germination percentage of *S. Zopilotensis* seeds of 70 and 273 dac under latency breaking treatments.

Treatments	Group (dac)	Mean
T 6 h	273	48.60 a*
T Bio	273	44.40 a
T 12 h	273	24.80 b
T 6 h	70	6.60 c
T 24 h	70	4.60 c
T 12 h	70	3.00 c
T 24 h	273	2.80 c
T 0 h	70	2.40 c
T Bio	70	2.20 c
T 0 h	273	2.00 c

* Means with different letters in the same column differ statistically according to the Tukey test ($P < 0.05$).

CONCLUSIONS

During the three evaluation dates, a constant behavior was registered in the treatments with six, 12 hours of soaking in water and six hours of soaking in Biozyme using seeds with 273 days of storage, the best combination to induce germination in "tuna pelona" was using seeds with 273 days of storage and apply the treatments soaking in water for 6 hours or soaking in the Biozyme TF® hormonal stimulant for 6 hours. It is necessary to extend the study with other mixtures of hormonal solutions and imbibition times in order to confirm what is proposed here; It is also suggested continuing the study in the growth phase to know the effect of the treatments on the development of the seedling.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

DATA AVAILABILITY

The data sets used or analyzed during the current study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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AUTHORS' CONTRIBUTION

Conceptualization: B.P.O., A.M.O., and T.R.R. Methodology and fund acquisition: A.M.O. and B.P.O. Formal analysis, writing, revision and edition: B.P.O., Y.D.T., and P.G.E. Supervision and project management: A.M.O., and T.R.R. All authors read and approved the final manuscript.

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