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EFFECT OF HYDRATION-DEHYDRATION CYCLES DURING GERMINATION IN SEEDS OF THE GENUS FEROCACTUS

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Abstract: The germination process for the plant is a risky stage during its life cycle, especially for those belonging to arid zones, such as cacti, which go through discontinuous periods of hydration, therefore, hydration memory is a process vital and a natural adaptation for these plants. In the present work the seeds of: *E. grusonii*, *F. clausen*, *F. peninsulae*, *F. pilosus*, *F. recurvus*; of which only the last of these evidenced this phenomenon by expressing germination curves similar to:*F. peninsulae*, plant that in 2014 the presence of this process was confirmed; temperature, in addition to water, was a limiting factor in the trials, by subjecting the species to different temperatures that affected germination to a greater extent as it decreased.
Keywords: *Ferocactus recurvus*, Germination, Hydration Memory.

INTRODUCCIÓN

Plants are a vital part of biodiversity, becoming an essential resource for the planet, having great economic, cultural, nutritional, medicinal importance, etc., worldwide; due to this they have had an anthropocentric development (SCBD, 2009). Mexico is a megadiverse country, in which 60% of its territory is arid or semi-desert, thus allowing the development of a large number of cacti, which is why it has more than half of the almost 2,000 varieties of cacti and succulents registered. before the Unesco World Network of Biosphere Reserves and a high index of endemism at the generic level (73%) and species (78%) (Hernández and Godínez, 1994). Among the present diversity of the cactaceae family we can highlight the columnar cacti (viejitos and tetechos); candelabriform cacti (cardones, organs and pitayas); the biznagas and biznaguitas (chilitos); some climbers like nopalillos (*Heliocereus* spp.); pitahayas (*Hylocereus* spp.), and a great variety of nopales, among others, referring to the area of the Tehuacán-Cuicatlán valley, Puebla (Jiménez, 2011).

Cacti, for being angiosperm plants, they present the formation, dispersal and germination of seeds, as fundamental events in the life cycle; acting with the function of multiplying and perpetuating the species; therefore, it is the main reproductive organ of the vast majority of higher plants (Doria, 2010). The seed is a complex reproductive unit, which in species from arid zones have developed strategies to germinate with little available moisture and prolonged droughts. It has been reported that seeds of some cacti belonging to the genus: *Ferocactus* respond to discontinuous humidity events ("hydration memory"), due to the fact that in some species the germination time is shortened after dehydration treatments compared to constant humidity treatments. As water is a limiting factor in arid environments, it plays a key role in plant germination (Evenari, 1985), which is why it is a risky transition process for the seed, especially in this type of dry environment when The periods of humidity received are not stable (Dubrovsky, 1996; Cervantes and Martínez, 2000; López et al, 2014; Contreras et al, 2015).

On the other hand, the propagation of plants by means of seeds is of great ecological importance because it intervenes to a large extent in the maintenance of the genetic diversity of the species (Dubrovsky, 1998), therefore, the present work has the purpose of to identify the effect of hydration-dehydration cycles in seeds of the genus: *Ferocactus*.

METHODOLOGY

The standardization of techniques for germination in seeds of: *E. grusonii* (Zacatecas, 2016), *F. clausen* (Zacatecas, 2018), *F. peninsulae* (Baja California, 2014), *F. pilosus* (Zacatecas, 2017), *F. recurvus* (Puebla, 2018);

establishing the elaboration of 3 tests: T=15°C with controlled photoperiod (12 hours day/night), T=25°C with laboratory photoperiod and T=35°C with controlled photoperiod (12 hours day/night); all with a constant relative humidity of 60-65%.

For each of the tests, 50 seeds were extracted from their fruit, separating them from the pulp to wash them, scarifying them with 30% sodium hypochlorite solution for 15 minutes and then rinsing with water until the seed was completely removed. solution; Two boxes were used per species (one corresponding to the hydration-dehydration (HD) treatment and the other to the Control) with 25 seeds each. The petri dish was previously covered with a layer of filter paper moistened with 6ml of distilled water, the seeds were prepared. boxes were sealed with egapack to prevent moisture loss inside.

SEED TREATMENT

The treatment was subjected to a single cycle of HD (hydration-dehydration) that consists of incubating them under germination conditions for 3 days, drying for 4 days in total darkness by wrapping the petri dish with aluminum and finally placing them back under conditions of germination. While the seeds corresponding to the control treatment always maintained the germination conditions.

The mean germination time was calculated with: ΣG_{ji}, ΣG_i

where: i is day one of germination and: G_i is the number of seeds germinated on day 1 (Dubrovsky, 1996).

SAMPLE SEPARATION

The samples were placed in eppendorf tubes to be frozen until RNA extraction according to the following times:

1. T. zero: After the seeds have been scarified;
2. T. dehydration: When the treatment was withdrawn from germination conditions;
3. T. hydration: The treatment was placed in germination conditions;
4. T. germination: The radicle measures half a centimeter.

RESULTS AND DISCUSSION

In the first test, the seeds that were subjected to low temperatures (15°C) did not generate the optimal conditions for germination, causing a slow development and the proliferation of fungi in the seeds, affecting their development, taking at least 3 weeks to germinate. They were not included in subsequent analyses. On the other hand, the test carried out at a temperature of 25° C presented the highest percentage of germination (Figure 1), however, no clear evidence of hydration memory was observed in the dehydration treatments when germinating in a greater range of time than expected (Table 1).

However, *F. recurvus* presented the best expected standard curve in relation to: *F. peninsulae,* as it was observed in Figure 1a when the HD treatment germinated 4 days earlier than the control seeds; *E. grusonii* was the species used as a negative control as it did not belong to the genus: *Ferocactus,* presented the expected results as its germination was affected by dehydration (Figures 2a and 3a). On the other hand: *F. clausen* maintained the same germination time with or without dehydration, but the number of germinated seeds was affected by 50% in relation to its control group; while: *F. pilosus* had a slight decrease in germination time after having the HD treatment but in the same way that: *F. clausen,* its percentage of germinated seeds was affected (Figure 2a and 3a).

The test carried out at 35oC showed a decrease in germination time in relation to what was observed in previous tests (Table

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	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Tg
1C										25											25
1 HD												8									8
2C								25													25
2 HD											5	5			2						12
3C											11	14									25
3 HD											5				8		2				15
4C										2	12	2			2		2				20
4 HD															1		5				6
5C											5				6		2				13
5 HD																	6				6

Table 1: Daily record corresponding to the 25°C test; blue indicates the hydration time, yellow dehydration, pink germination and green the total germination. For *F. pilosus* (1C and 1HD), *F. clausen* (2C and 2HD), *F. recurvus* (3C and 3HD), *F. peninsulae* (4C and 4HD) and *E. grusonii* (5C and 5HD).

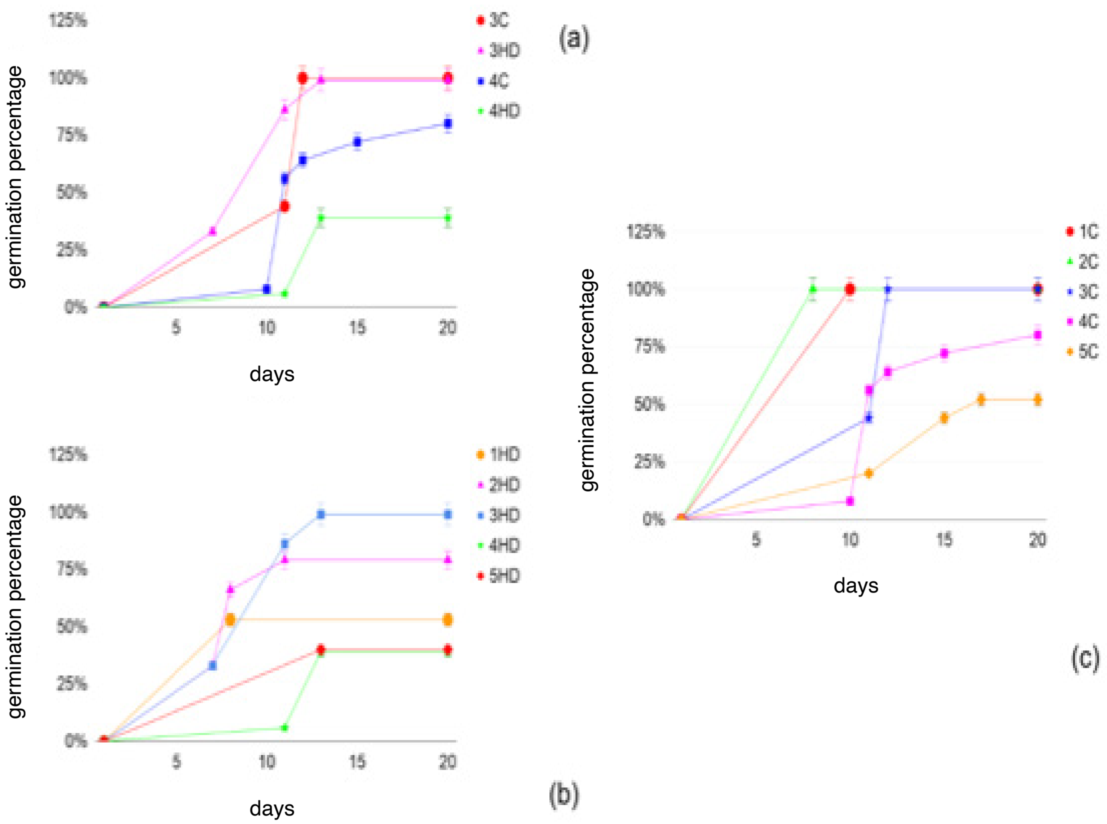

Figure 1. Effect on germination in the 25°C trial, (a) Comparative germination curves of: *F. recurvus* (3C y 3HD) and *F. peninsulae* (4C y 4HD) for Control and HD samples. (b) Comparative germination curves of all HD treatments; in orange to observe:*F. pilosus,* in pink: *F. clausen,* in blue: *F. recurvus,* in green: *F. peninsulae and in red*: *E. grusonii* (c) Comparative germination curves of all control treatments; in red color of notes:*F. pilosus,* in green: *F. clausen,* in blue: *F. recurvus,* in pink: *F. peninsulae and in orange*: *E. grusonii.*

1). Unfortunately, the inconvenience was that the sprouter used burned the boxes due to its proximity to the resistance that generated heat., thus ending the trial on its 13th day.

At this temperature the greatest decrease in germination time is observed, being more evident for: *F. recurvus,* when germinating earlier and 10% more than the control seeds (Figure 2b and 2c), therefore, comparing them with: *F. peninsulae* (Figure 2a) a hydration memory process is presumptively observed, therefore, it must be corroborated by a genomic analysis.

On the other hand, *F. pilosus* with this temperature there was a drastic decrease in the germination percentage for the seeds that were dehydrated, as well as the decrease of the same (Figure 2b and 2c); *F clausen* presented a decrease in germination time, but its percentage of germinated seeds was also affected by almost 50% (Figure 2b and 2c), so these species will not be considered for a subsequent genomic analysis.

Finally, with the data obtained, the average germination time was calculated in which: *E. grusonii* obtained the highest values compared to the other cacti; The seeds that had a shorter average germination time were: *F. pilosus*, while *F. recurvus* y *F. peninsulae* presented intermediate data (Figure 3).

Trials at different temperatures showed optimization at 35°C, but according to Castillo in 1986, F. histrix presents better germination at 25°C; while Flores et al in 2017 and Pérez et al in 2011 for the same species observed that at 30°C the germination percentage increases, so it can be observed with these variations that Ferocactus seeds are very sensitive to changes in the temperature, increasing the germination time as they are not in optimal conditions, as was observed when subjecting them to 15°C, added to this once the seed has germinated, if low temperatures are maintained, the seedling is highly exposed to fungal infection and to the deterioration of the root because of them.

	1	2	3	4	5	6	7	8	9	10	11	12	13	TG
1 C								2		9				11
1 HD													1	1
2 C							8	8	1	1				18
2 HD										2			3	5
3 C							4	3	3	1				11
3 HD										1			6	7
4 C							1			1				2
4 HD													5	5
5 C								2						2
5 HD														0

Table 2: Daily record corresponding to the 35°C test; blue indicates the hydration time, yellow dehydration, pink germination and green the total germination. For *F. pilosus* (1C and 1HD), *F. clausen* (2C and 2HD), *F. recurvus* (3C y 3HD), *F. peninsulae* (4C and 4HD) and *E. grusonii* (5C and 5HD)

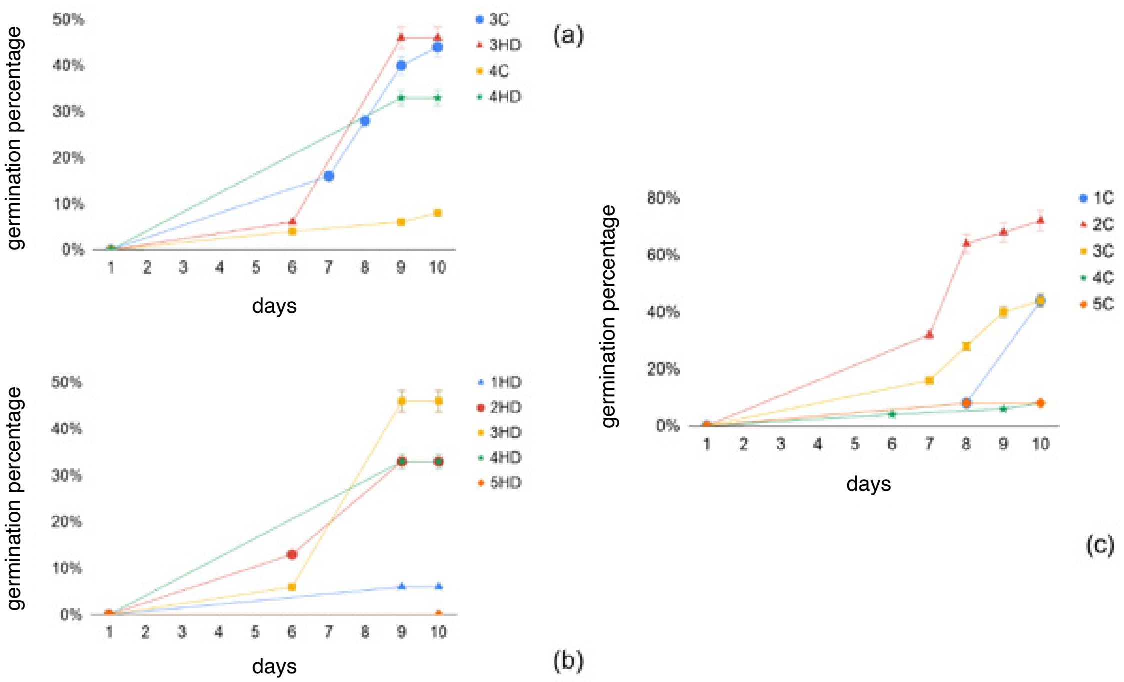

Figure 2. Effect on germination in the 35°C trial, (a) Comparative germination curves of:*F. recurvus* (3C y 3HD) and *F. peninsulae* (4C y 4HD) for Control and HD samples. (b) Comparative germination curves of all HD treatments; in blue color of observe *F. pilosus,* in red: *F. clausen,* in yellow: *F. recurvus,* in green: *F. peninsulae and in orange*: *E. grusonii*. (c) Comparative germination curves of all control treatments; in blue color of observe: *F. pilosus,* in red: *F. clausen,* in yellow: *F. recurvus,* in green: *F. peninsulae and in orange: E. grusonii.*

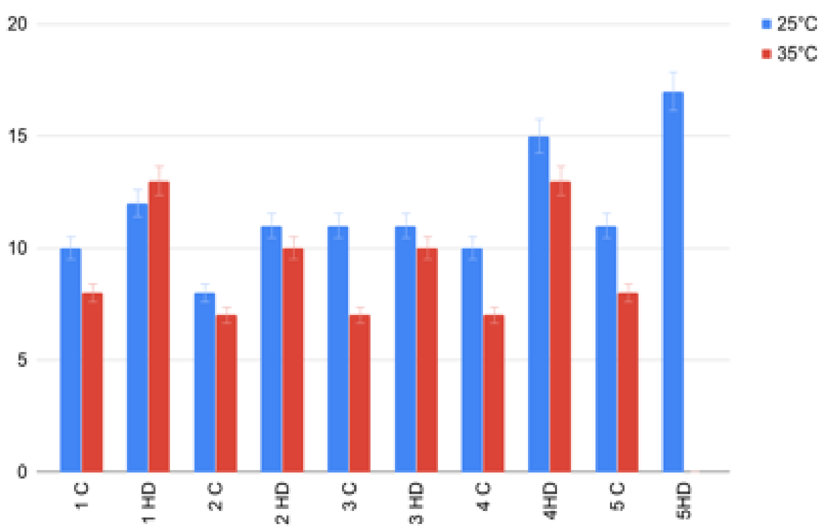

Figure 3. Mean germination time expressed in days for: *F. pilosus* (1C and 1HD), *F. clausen* (2C y 2HD), *F. recurvus* (3C y 3HD), *F. peninsulae* (4C y 4HD) and *E. grusonii* (5C y 5HD).

Scarification is an important step for the in vitro germination of seeds, since it allows the softening of the testa and the permeability to hydration and thus allows the activation of the embryo present in the seed, previous studies such as the one carried out by Barrera et al in 2014 and Lima-Meiado in 2018, highlight the importance of scarification, because once it is done, the seeds improve germination and the radicle presents greater vigor in its early stages of growth; In addition to this, a correct scarification removes from the seed some bacteria and fungi that may affect the development of the seedling in its early stages.

The scarification used in this work is mainly focused on the sterilization of the outside of the seed, so it is necessary to incorporate into the methodology techniques that allow a complete scarification and that seeks to simulate the natural conditions that the seed faces, such as It was carried out by Alvarez and Montaña in 1997 by subjecting seeds of *F. latispinus* a scarification that simulated the digestion that occurred in the digestive tract of birds (chemical scarification) and the natural drag that the seed would have when it falls to the ground generated by the air (mechanical scarification).

Dubrovsky in 1996 with seeds of *Stenocereus* observed that some species have the ability to conserve, during temporary dehydration, physiological changes such as protein expression, induced by seed hydration. This process is known as hydration memory and can be replicated in vitro by subjecting the seed to HD treatments or cycles. Regarding these treatments, López et al., in 2014 standardized cycles of 4 days of hydration and 3 days of constant dehydration that were used in the present work, with the modification of Dubrovsky in 1996 by stressing the sedes, during dehydration, this in order to make the expected effect more noticeable, having an average germination of 10 days, but Santini et al., in 2017 in seeds of: *Ferocactus* show an average of 7 days when having cycles of 4 days of hydration and 1 day of dehydration, so it is necessary to carry out further work to know the most optimal conformation of the HD cycles for seeds of: *Ferocactus*.

The modification of darkness made to the HD cycles allows, as previously mentioned, that the hydration memory effect is more evident, but the longer the time in the dark, the seed presents more complications to germinate.

As it was explained by Romero et al., in 1992 when submitting to: *F. peninsulae* under different stress conditions, germination stops completely when the seed is left in the dark for a long time, in addition to which germination is also reduced when the seed is left without water for large amounts of time (1 month), so it is necessary to be careful when performing the treatments.

Of the 3 problem plants used in this work only in: *F. recurvus* the pattern was observed with greater similarity to: *F. peninsulae,* which in 2014 López et al determined that it presents the phenomenon of hydration memory, so it is highly probable that: *F. recurvus* in the same way the present, for which it is necessary to carry out a profile of genomic expression to corroborate these results.

F. recurvus is especially important since, according to Martínez et al in 2017, this is a species with multiple uses in communities of Oaxaca, among which edible, ceremonial and ornamental use stands out, for which it is cultivated by its inhabitants and this process of hydration memory could improve its production, as well as reduce the amount of water necessary for its germination and development.

The effect of HD cycles in cactaceous plants allows the hydration memory effect to occur, so this is a special adaptation naturally presented to these plants to survive the adverse conditions present in their environments; this is corroborated by Contreras et al., in 2005 by subjecting seeds of non-cactaceous plants to these in vitro cycles, thus observing that germination does not improve.

In addition, this process is especially important for cactaceous plants, as Armador et al observed when comparing the effect it has in relation to different growth regulators such as AIA, ANA and AG; determining that the best development is observed in seeds that undergo HD cycles.

CONCLUSIONS

Temperature is an important factor for seed germination, noting that the best germination is between 25°C and 30°C depending on the species to work with.

The HD cycles do improve the germination of certain seeds of *Ferocactus*, being a natural process in the seed.

F. pilosus y *F. clausen* do not present the hydration memory process as their germination was not improved after having an HD cycle compared to the control group.

F. recurvus presented the best expected standard curve based on: *F. peninsulae,* therefore, further work is necessary.

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