Short Note [Nota Corta]

In vitro GERMINATION AND INDUCTION OF MORPHOGENETIC RESPONSES IN PITAHAYA (*Hylocereus undatus* (HAW.) BRITTON & ROSE) †

[GERMINACIÓN *in vitro* E INDUCCIÓN DE RESPUESTAS MORFOGENÉTICAS EN PITAHAYA (*Hylocereus undatus* (HAW.) BRITTON & ROSE)]

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SUMMARY

Background. *Hylocereus undatus* is a plant of great economic importance that produces an edible fruit known as Pitahaya or dragon fruit. However, these methods result in a reduced genetic base and low germination rates, in addition to the low germination of seeds, which makes successful cultivation difficult. **Objective.** To establish an efficient procedure for disinfecting and germinating *H. undatus* seeds, laying the groundwork for future genetic improvement programs. Methodology. *In vitro* conditions, cladodes, cotyledons leaves, and stems were used as explants and cultured in MS medium with varying concentrations of ANA and BAP (0.5 mgL^{-1}, 1.0 mgL^{-1}, 1.5 mgL^{-1}, 2.0 mgL^{-1}, 2.5 mgL^{-1}). **Results.** An 86% germination rate using MS medium without sucrose and a 47% germination rate in MS medium with added sucrose (30 gL^{-1}). In the induction stage of morphogenetic responses, it was observed that the highest proliferation and shoot formation occurred when ANA was added at a concentration of 2.0 mgL^{-1} in the MS medium. **Implications**. It is necessary to lay the foundations for future genetic improvement programs aimed at increasing the plant material available to Pitahaya producers in Mexico. **Conclusions**. The best responses were seen in the cladodes, resulting in a greater number of shoots per explant.

Key words: cacti; in vitro culture; morphogenesis.

RESUMEN

Antecedentes. *Hylocereus undatus* es una planta de gran importancia económica que produce un fruto comestible conocido como Pitahaya o la fruta del dragón. Es tradicionalmente propagada por medio de semillas o esquejes. Sin embargo, estos métodos de propagación generan reducción de la base genética, además de la baja germinación de las semillas que dificulta el éxito del cultivo. **Objetivo**. Establecer un procedimiento eficiente para la desinfección y germinación de las semillas de *H. undatus* para sentar las bases en futuros programas de mejoramiento genético. **Metodología**. Se usaron cladodios bajo condiciones *in vitro*, hojas cotiledóneas y tallos como fuente de explante, en medio MS con diferentes concentraciones de ANA y BAP (0.5 mgL^{-1}, 1.0 mgL^{-1}, 1.5 mgL^{-1}, 2.0 mgL^{-1}, 2.5 mgL^{-1}). **Resultados**. El porcentaje de germinación fue del 86% utilizando medio MS sin sacarosa y el 47% de germinación en

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medio MS adicionado de sacarosa (30 gL^{-1}). En la etapa da inducción de respuestas morfogenéticas de *H. undatus*, se observó que la activación de los explantes se llevó a cabo en medio MS adicionando ANA con 2.0 mgL^{-1}, con esta concentración se dio la mayor proliferación y formación de brotes. **Implicaciones**. Es necesario sentar las bases para futuros programas de mejoramiento genético, encaminados a incrementar el material vegetal disponible para los productores de Pitahaya en México. **Conclusiones**. Las mejores respuestas se dieron en los cladodios, al obtener el mayor número de brotes por explante.
Palabras Clave: cactáceas; cultivo *in vitro*; morfogénesis.

INTRODUCTION

Pitahaya (*Hylocereus undatus*) is classified within the cactus family, which is natively distributed in the American Continent (Caetano-Nunez *et al*., 2014). Pitahaya is an epiphytic and climbing plant (Caetano-Nunez *et al*., 2014). *H. undatus* and *H. polyrhizus* are two species of great importance because they produce edible fruits. These species are considered an excellent food resource due to their high nutritional, cosmetic, and medicinal value; additionally, *H. undatus* has positioned itself in recent years as a viable alternative for ornamental and fruit cultivation with great profit potential for small agricultural producers, which leads to good acceptance in the international market (Trindade *et al*., 2023; Trivellini *et al*., 2020).

In Mexico, the edible species with the greatest natural distribution is *H. undatus.* Its main plantations are found in the states of Quintana Roo, Yucatan and Puebla (SIAP, 2023). Pitahaya is propagated mainly by seeds and cuttings, methods that are inefficient and slow due to the high susceptibility of the species to biotic and abiotic factors, such as humidity, drought, salinity, and diseases caused by various pathogens, respectively. In addition, the problem is increased by the low viability of the seeds to germinate in field conditions (Hua *et al*., 2015). On the other hand, Pitahaya plants obtained from seeds show a juvenile phase that exceeds four years where the production of flowers and fruits is not progressive and the plants obtained are practically hybrids (Livera-Muñoz *et al*., 2010; Trindade *et al*., 2019).

Another important aspect to point out is that this crop is relatively new, so most producers are unaware of the optimal and adequate management of it. For these reasons, it is necessary to use biotechnological techniques that guarantee the obtaining of disease-free plants, preserving their genetic vigor, but with outstanding sanitary quality with the perspective of satisfying the demands of plant material in commercial plantations (Montesinos-Cruz *et al*., 2015; Guzmán-López and Salinas-Castro, 2022). Plant biotechnology and *in vitro* culture of plant tissues offer a series of techniques that can be incorporated into the conservation schemes of plant genetic resources, through mass propagation techniques and obtaining certified disease-free plants, under controlled conditions, quickly and through faster processes, at low cost and in reduced spaces are achieved efficiently and effectively (Pérez-Molphe- Balch *et al*., 2012). The objective of this work was establish an efficient procedure for disinfecting and germinating *H. undatus* seeds, laying the groundwork for future genetic improvement programs.

MATERIALS AND METHODS

Plant material

The plant material used in this work was obtained from mature fruits of *H. undatus*, from "Xocuitlan de Todos Santos", Puebla, Mexico. To obtain the seeds, the fruit was washed with detergent and water to eliminate contaminants. The seeds were then removed from the mesocarp using a manual strainer and the newly extracted seeds were washed with running water. The seeds were left to dry for 24 hours in a cool area with sufficient ventilation.

In vitro disinfection protocol

200 *H. undatus* seeds were used. The first wash was carried out with running water to remove the mesocarp and then they were transferred to a container with sterile distilled water. They were then washed with 70% (v/v) ethyl alcohol for 1 minute and placed in a 30% (v/v) sodium hypochlorite solution for 20 minutes with constant agitation. Finally, three rinses were carried out with sterile distilled water in a laminar flow hood and the seeds were sown in *in vitro* culture media.

In vitro establishment of seeds

After disinfection, the seeds were sown in Murashige and Skoog (1962), (MS) culture media with 30 gL^{-1} of sucrose and without sucrose, 2.5 gL^{-1} of Phytagel to gel. The flasks with the medium were sterilized in an autoclave at 121°C at 15 pounds of pressure for 15 minutes, then allowed to solidify at room temperature in a sterile place. The seed germination conditions were as follows: photoperiod 24 hours with light and temperature of 25±1°C during crop development.

Effect of ANA and BAP on the induction of morphogenesis

In the induction of morphogenetic responses, seedlings obtained from germinated seeds were used, from

which they were sectioned into three parts: cladode, cotyledonary leaves, and stem for the induction of morphogenetic responses. Each explant was sown in an MS culture medium, with different doses of ANA (Naphthaleneacetic acid) and Benzyl-aminopurine (BAP) (0.5, 1.0, 1.5, 2.0, and 2.5 mgL^{-1}) for both growth regulators. In all the treatments the pH was adjusted to 5.8.

The culture media were distributed in a completely random design, under controlled conditions of 24 hours photoperiod with light, by white light lamps and a temperature of 25±1°C during the development of the culture. Six months after the induction period, the morphogenetic response was evaluated by counting the number of shoots per explant for each growth regulator used.

Experimental design

For the germination test, a completely randomized design was established, considering the factors of MS culture media with sucrose (30 gL^{-1}) and MS medium without sucrose. Ten seeds were placed in each flask, with 10 repetitions for a total of 100 seeds per medium. The response variable was the percentage of germination. The factors analyzed were the type of regulator (ANA and BAP) and dose (0.5, 1.0, 1.5, 2.0, and 2.5 mgL^{-1}), for a total of 11 treatments. The response variable: number of shoots per type of explant.

Statistical analysis

For the germination test, a *t*-test was performed, while for the effect of the growth regulators, the data, as they did not present normality, were analyzed through the Kruskal-Wally's test, in the Statistical Version 7 software.

RESULTS

In vitro germination

The method used achieved 90% of seed disinfection, which is one of the most important steps for stablishing the crop. Seed germination began 4 days after sowing, with a higher percentage of germination observed in the medium without the addition of saccharose. In MS medium without saccharose, 86% of germinated seedlings were observed, with a larger size and a thicker stem, see (Fig. 1). The root system developed a greater quantity of absorbent hairs, this being one of the first morphogenetic responses, while in the containing medium saccharose, there was a 47% germination with seedlings of smaller size but with much fewer roots. These results were corroborated by obtaining significant differences, with the *t*-test applied (t= -5.96287; p-value= 0.00001622) comparing mediums.

Figure 1. Morphological structures of *Hylocereus undatus* seeds germinated in MS medium without saccharose.

Effect of growth regulators

The Kruskal-Wallis test showed that there are no significant differences between the evaluated treatments. Shoots and calluses were obtained as the main morphogenetic responses a concentration of 2.0 mgL^{-1} of ANA. The most relevant response was observed in the apical part of the stem, where the largest number of shoots were formed with greater size and thickness. On the other hand, in the explants sown with ANA as a growth regulator, at a concentration of 0.5 mgL^{-1}, the stems and leaves began to generate absorbent hairs as the dose of this growth regulator increased. At the stem level, oxidation occurred at the edges of the explants.

The effect of growth regulators with BAP in the different concentrations (0.5, 1.0, 1.5 mgL^{-1}), presented very few changes, one of them occurred at the edges of the explants where oxidation occurred when increasing the concentration of the growth regulator from 2.0 to 2.5 mgL^{-1}; with this last concentration, some more noticeable changes occurred at the end of the test, with wider leaves, callus formation, and quite reduced rhizogenesis, and small apical shoots, but no lateral roots were generated in the stems. Additionally, by increasing its concentration, the formation of absorbent hairs was achieved, although in low quantities.

When evaluating the types of explants, no statistical differences were obtained either, however, a trend was

observed about a greater number of shoots when using cladodes as a source of explant to induce morphogenetic responses, from which an average of 10.7 shoots were obtained per cladode used in this stage of the work. Thus, it can be considered that the best explant for the formation of new shoots and roots were the cladodes with the addition of ANA at a concentration of 2.0 mgL^{-1}, with a stimulating effect on the formation of 4 to 5 shoots per explant (Figs. 2. **a** and **b**).

DISCUSSION

Seed germination in medium with saccharose was very low, possibly because a higher concentration of solutes in the medium increases the osmotic potential, preventing the seed from absorbing water. Sugars and macronutrients affect the osmotic potential of the MS culture medium (George, 1993; Yoshida *et al*., 1973). Furthermore, Cárdenas-Lara and Villegas-Monter (2002) found that the osmotic potential became more negative with an increase in the sucrose concentration in the medium. This could be the reason for the low germination percentage in the medium with saccharose compared to the medium without this carbon source.

Disinfection of the material (explants or seeds) is an important step to avoid and reduce the risks of in vitro contamination. Reports by Roca and Mroginski (1993) standardized the use of sodium hypochlorite (NaClO) at concentrations of 1 to 3% as the most efficient agent as a germicide in seeds and plant tissues to eliminate bacterial and fungal agents. On the other hand, some authors indicate that the use of distilled water can be managed, followed by 70% alcohol for 30 seconds and 1% chlorine for 10 minutes alternating with distilled water rinses (Montiel-Frausto *et al*., 2016; Moreira-Palacio and Sánchez-Rodriguez, 2017). Our results showed adequate establishment of germination media with high percentages of disinfection (90%), this indicates that the procedure is optimal for *H. undatus*.

The results obtained in the multiplication stage indicate that the use of ANA as a plant growth regulator promotes rhizogenesis, stem elongation, as well as the formation of new shoots. Some micropropagation studies of pitahaya have reported that the combination of concentrations of cytokinins and auxins, are essential for shoot multiplication and promoting root formation in vitro (Fan *et al*., 2013; Trivellini *et al*., 2020). However, spontaneous rhizogenesis of pitahaya has been achieved when vitro seedlings are subcultured in media without growth regulators or with low concentrations of these regulators (De Medeiros *et al*., 2006; Viñas *et al*., 2012).

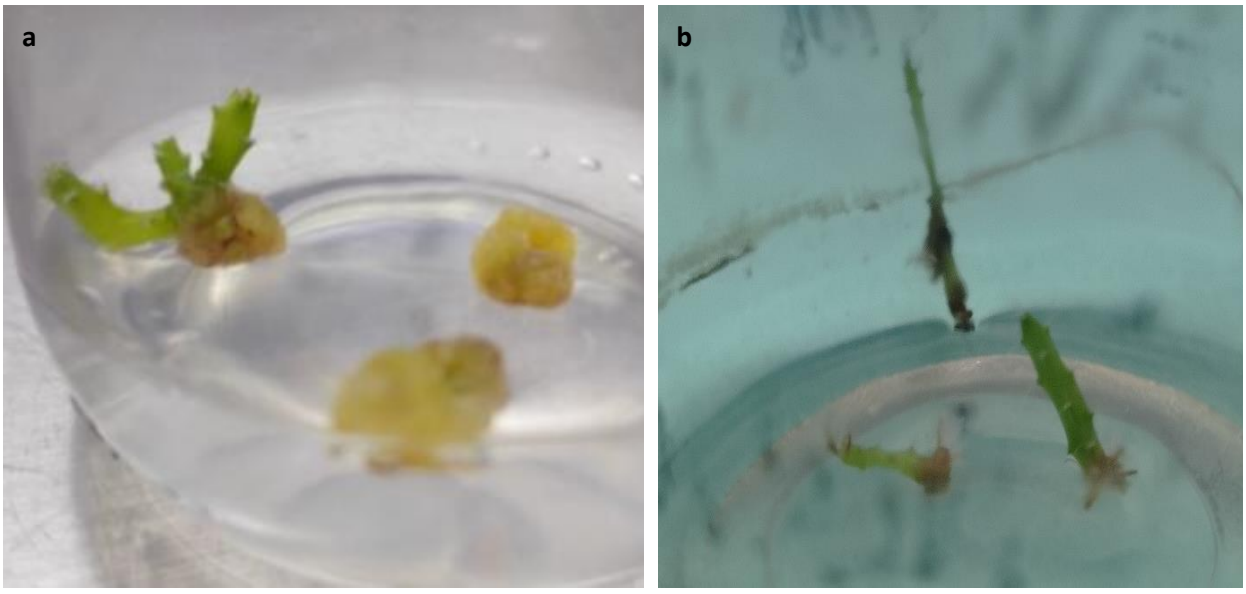

Figure 2. Effect of growth regulators ANA on *Hylocereus undatus* (**a.** thorns, areolas. **b.** roots hairs) shoots and calluses.

CONCLUSIONS

An aseptic culture was established with a 90% successful disinfection percentage. Likewise, the germination percentage was determined at 86% with MS without saccharose. The explant with the greatest response potential is the cladode with ANA as a growth regulator and a concentration of 2.0 mgL^{-1}. Is necessary lay the foundations for future genetic improvement programs, aimed at increasing the plant material available to *H. undatus* producers in Mexico.

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Conflict of interest. None.

Compliance with ethical standards. Do not apply.

Data availability. Data are available with Nadia Guadalupe Sánchez-Coello (nasanchez@uv.mx).

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