Investigation note

Germination of orchids using a simple and economical method, reproducible in non-optimal environments

Citlalli Harris Valle§
Itzel Landero Benavidez
José Francisco Alvarado Vázquez
René Hernández Gómez

[1]Zacapoaxtla Higher Technological Institute. Highway Acuaco-Zacapoaxtla km 8, Col. Totoltepec, Zacapoaxtla, Puebla, Mexico. ZC. 73680. (itzi22.ilb@gmail.com; ialvarado972fra@gmail.com; hdzrene@hotmail.com).

§Corresponding author: citlalliharris@yahoo.com.mx.

Abstract

The cultivation of orchids is complicated due to the low germination rate of the seeds, as well as the difficulties in the survival and establishment of the plants. It is known that these plants require to be associated with endophytic fungi (they colonize the roots and facilitate the nutrition processes of plants). The foregoing causes people engaged in the trade in orchids as ornamental plants illegally extract wild specimens from preserved forests. This paper proposes a strategy of germination of orchids using economic and accessible elements, which can be carried out in places without laboratory infrastructure. The basic elements are clear glass jars, a cardboard support, water and a drawer adapted to maintain a sterile environment. The strategy is functional in terms of its viability to develop in environments not optimal for this type of work, germinated seeds were obtained but it is necessary to determine the right time and find a way to extract the germinated seeds from the jars and place them in substrates that allow their establishment and development. The number of germinated seeds is low; however, it is considered that it will serve as a basis for non-specialized people to propagate orchids in their localities from the germination stage.

Keywords: economic procedure, ornamental plants, reproduction.

Reception date: May 2021
Acceptance date: June 2021

The propagation of orchids is complicated due to the characteristics of this group of plants. It is documented that its germination depends on the environmental conditions in which the seed is found, the fertilization rate and the presence of the symbiont microorganisms (Arditi *et al*., 2000; Karol *et al*., 2015). Likewise, the establishment of seedlings and their development to adult individuals is complicated, mainly in epiphytic specimens (Damon, 2003).

There are some strategies of reproduction of orchids, however, the most successful methods are expensive due to the requirements (development conditions, *in vitro* culture media, use of phytohormones, specialized materials, equipment, etc.) and special infrastructure is needed (Suzuki *et al*., 2012; Pradhan *et al*., 2016). Due to the above, it is common for people engaged in the trade in orchids as ornamental plants to extract specimens from conserved forests, generally the extraction is illegal causing the wild populations to be damaged, even some species are already in some category of conservation within NOM-059-2010 (Hágsater *et al*., 2005).

In the northeastern region of Puebla, there are important populations of the family Orquidiacea, the geographical conditions of the region allow the diversity of this type of plants to be high (Pérez Bravo *et al*., 2010). In addition, the tourist vocation of the area causes visitors to look for and acquire orchids and therefore they are well priced (Emeterio-Lara *et al*., 2016). It is difficult to counteract or eliminate the commercialization of illegal specimens, because they constitute a source of income for many families (Cruz *et al*., 2015).

A strategy that the inhabitants of these sites have is to propagate them by fragmentation using bulbs or cuttings; however, it is rare. In this work, a strategy of germination of orchids was standardized through a simple and economical method, which can be reproducible by people from rural communities that have the appropriate climatic conditions for the development of these plants. It seeks to lay the foundations for the development of a propagation strategy as an alternative for families engaged in the trade in ornamental plants, that is economically accessible.

The first step was the elaboration of the germination systems using transparent glass jars of 100 to 150 ml capacity, inside the bottle, cardboard cones of material used for egg transport were placed, the mouth of the bottle was covered with sterile gauze and fixed with bands (Figure 1a). For planting, a homemade chamber was designed (using boxes lined internally with aluminum) for the maintenance of a sterile environment, with lit candles as a way to replace the use of Bunsen burners (Figure 1b). The bottles were disinfected in bain-marie.

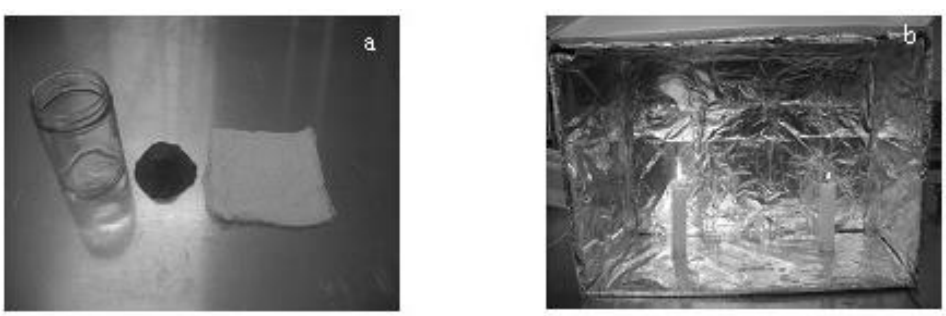

Figure 1. Materials used for the elaboration of the container that functioned as a seed germination system (a) and a drawer that allowed maintaining the sterile environment (b).

The sowing of the seeds was done inside the chambers, the seeds were wrapped in folded brown paper as an envelope and disinfected by placing the envelops in a 5% chlorine solution for one minute and then transferred to a 10% alcohol solution for 30 seconds, finally they were rinsed with boiled water (Figure 2a).

Before placing the seeds inside the jar’s, boiled water was added to the disinfected jar, inside the sterile environment, covering the base of the cardboard cone at a height of approximately 1 cm, then the paper envelopes were opened, and the seeds were placed at the top of the cones taking with tweezers the inverted paper and repeatedly touching the surface of the cardboard as if it were sprinkling (Figure 2b). A total of 39 jars were prepared, using seeds of three different species (13 of each): *Gongogra* sp., *Vanillan planifolia*, *Epidendrum* sp., collected in the forests of the Zacapoaxtla region.

Figure 2. Seed disinfection procedure prior to sowing (a) method of sowing (b).

The bottles were placed in site with natural lighting, with little insolation and inside closed plastic boxes to avoid dust contamination. Changes in the seeds were monitored using a stereoscopic microscope to detect swelling or germination. The samples were reviewed weekly, when the water level decreased boiled, water was added with a sterile syringe.

The procedure for the elaboration of the germination systems was simple and practical, 13 jars were contaminated and discarded. So far, germination was observed only in seeds of *V. planifolia* was observed (Figure 3a), while in *Gongogra* sp., only swollen seeds were observed (Figure 3b).

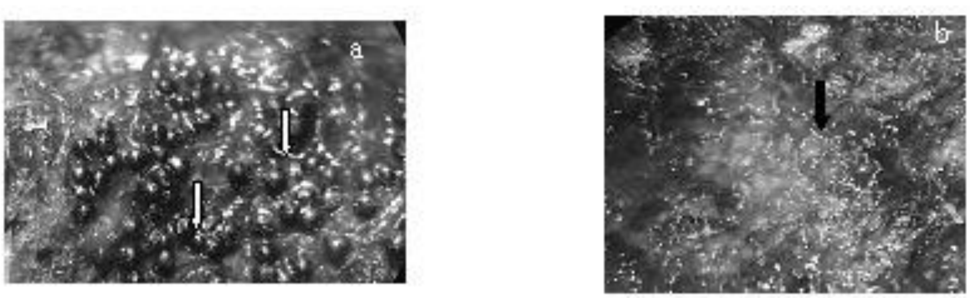

Figure 3. Seeds of *Vanillan planifolia* (a); and *Gongogra* sp. (b), after 180 days of placement in the germination system, observed under a 4x stereoscopic microscope. The white arrows indicate the expulsion, in the seeds, of tissue that will become roots, the black arrow points to the seeds.

Swelling was observed in all seeds; however, so far, only the expulsion of tissue that will constitute the root has been detected in three of the 13 jars in which seeds of *V. planifolia* were placed. The germinated seeds were transferred to a synthetic culture medium to verify their viability, but they have not been successfully transferred. It is believed that the transfer was made prematurely, causing the seedlings not to continue their growth or to become contaminated. So far, there is no functional strategy for the second stage of culture.

Strategies have been developed for the germination of orchid seeds that include the use of symbiotic fungi; however, it is not yet clear what is the best way to obtain successful results in different growing conditions (Pereira *et al*., 2005; Otero *et al*., 2013; Mercado *et al*., 2020). Otero and Bayman (2009) point out that the most effective methodology is under asymbiotic conditions; however, for this case, it is considered necessary to search for associated fungi in wild plants that stimulate the germination of seeds. There are even studies that indicate that a pretreatment of the seeds allows the percentages of viability and germination to increase by more than 90% (Salazar Mercado *et al*., 2019).

On the other hand, there is the possibility of using organic products that serve as chemical signals (banana pulp or coconut water), whose content of sugars, amino acids, antioxidants, minerals, organic acids, etc., promote plant growth (Arditi, 1993; Moreno and Menchaca, 2007). Cheng *et al*. (2015) found that the addition of substances facilitates the germination and development of the embryo, however, the concentration and time in which the seeds remain in contact with these substances must be taken care of.

Tejeda-Sartorius *et al*. (2017) propose that, to grow orchids, the right specie must be chosen, and specific environmental conditions must be generated to develop them, so that it is considered that ecophysiological studies of plants should be the basis of the proposed strategies, bearing in mind the search for accessible procedures that can finally be reproducible by people who are dedicated to the trade in plants of the Orquidaceae family.

It is necessary to design strategies for the conservation of orchids in the conserved forests of Mexico, seeking a sustainable and fair management with the inhabitants of the places (Martínez-Feria, 2010) so this work aims to develop an economic and easily accessible procedure that is profitable for those who are dedicated to the orchid trade, thus reducing the impacts of illegal extraction of specimens.

Conclusions

The germination system works in general terms, but its efficiency is low, it is considered that an additional element should be sought such as the use of different endophytic fungi or applying stimulating extracts (containing phytohormones such as coconut water). It is essential to develop strategies that allow the transfer of the germinated seeds to a medium or substrate that allows them to continue with its development to achieve the establishment of the seedlings.

Cited literature

Arditti, J. and Ghani, K. A. 2000. Numerical and physical properties of orchid seeds and their biological implications. New Phytol. 145(3):367-421.

Chen, Y.; Goodale, U. M.; Fan, X. L.; and Gao, J. Y. 2015. Asymbiotic seed germination and in vitro seedling development of *Paphiopedilum spicerianum*: an orchid with an extremely small population in China. Global Ecol. Conserv. 3:367-378. Doi: 10.1016/j.gecco.2015.01.002.

Damon, A. 2003. Las epífitas. Ecosistemas y comunidades: procesos naturales y sociales de los bosques. Ecofronteras. 18:17-20.

Emeterio-Lara, A.; Palma-Linares, V.; Vázquez-García, L. M. and Mejía-Carranza, J. 2016. Usos y comercialización de orquídeas silvestres en la región sur del estado de México. Polibotánica. 42:197-214.

Hágsater, E.; Soto-Arenas, M. A.; Salazar-Chávez, G. A.; Jiménez-Machorro, R.; López-Rosas, M. A.; Dressler and Robert L. 2005. Las orquídeas de México. Instituto chinoin. México, DF. 304 p.

Karol-Chávez, H.; Mosquera-Espinosa, A. T. and Otero-Ospina, J. T. 2015. *In vitro* propagation of *Comparettia falcata* Poepp and endl. (Orchidaceae) seeds using symbiotic and asymbiotic techniques. Acta Agron. 64(2):125-133.

Martínez-Feria, A. 2010. Patrones de distribución y áreas prioritarias para la conservación de las orquídeas en riesgo de Oaxaca. Tesis de Maestría. Centro interdisciplinario de investigación para el desarrollo integral regional unidad Oaxaca. 67 p.

Mercado, S. A. S.; Caleño, J. D. Q. and Rozo, L. Y. M. 2020. Improvement of the methodology of the tetrazolium test using different pretreatments in seeds of the genus epidendrum (Orchidaceae). J. Seed Sci. 42(e202042013): 1-8. http://dx.doi.org/10.1590/2317-1545v42231028.

Moreno, D. and Menchaca, R. A. 2007. Efecto de los compuestos orgánicos en la propagación in vitro de stanhopea tigrina bateman orchidaceae. Foresta Veracruzana. 9(2):27-32.

Otero, J. T. and Bayman, P. 2009. Symbiotic *vs* Asym-biotic seed germination in epiphytic orchids. Acta Agron. 58(4):270-276.

Otero, J. T.; Mosquera-Espinosa, A. T. and Flanagan, N. S. 2013. Tropical orchid mycorrhizae: potential applications in orchid conservation, comercialization, and beyond. *In*: fourth scientific conference on andean orchids. Lankesteriana. 13(1-2):57-63.

Pérez-Bravo, R.; Salazar-Gerardo, A. and Mora-Guzmán, E. 2010. Orquídeas de las lomas-la manzanilla, Sierra Madre Oriental, Puebla, México. Boletín de la Sociedad Botánica de México. 87:125-129.

Pradhan, S.; Regmi, T.; Ranjit, M. and Pant, B. 2016. Production of virus-free orchid cymbidium aloifolium (L.) Sw. by various tissue culture techniques. Heliyon. 2(10):e00176. http://dx.doi.org/10.1016/j.heliyon.2016.e00176.

Salazar-Mercado, S. A.; Botello-Delgado, E. A. and Quintero-Caleño, J. D. 2019. Pre-treatments effect on the tetrazolium test on epidendrum barbaricum hágsater and dodson seeds. Acta Agron. 68(4):306-311. Doi: 10.1590/2317- 1545v42231028.

Suzuki, R. M.; Moreira, V. C.; Pescador, R. and Melo, F. W. 2012. Asymbiotic seed germination and in vitro seedling development of the threatened orchid *Hoffmannseggella cinnabarina*. *In vitro* Cellular and Developmental Biology-Pant. 48(5):500-511 Doi: 10.1007/s11627-012-9460-1.

Tejeda-Sartorius, O.; Téllez-Velasco, M. A. A. y Trejo-Téllez, L. I. 2017. Características ornamentales de orquídeas silvestres y su propagación con fines comerciales. Alternativa de aprovechamiento sustentable ex situ. Agroproductividad. 10(6):37-46.