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ORCHIDS' MICROPROPAGATION FOR TO THE SUSTAINABLE MANAGEMENT OF NATIVE SPECIES FROM PARQUE NACIONAL Y ÁREA NATURAL DE MANEJO INTEGRADO COTAPATA (PN-ANMI COTAPATA), LA PAZ-BOLIVIA

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Introduction

Bolivia is one of eleven countries with the highest biodiversity in earth, due to its variety of ecological belts, ecotones, biogeographic affinities, heterogenic habitats and total species number (Ibish 1996). Concerning to flora, approximately 20,000 angiosperms species have been registered (Beck 1998) and 1,500 of them are included in the *Orchidaceae* family. The region with the highest orchid diversity corresponds to the Yungas Mountain Forest which covers 4% of the national extension and has 60% of the species, being 80% of them endemic of the zone (Vásquez, 2004). In Bolivia, this group is considered as a priority for conservation since many species have some degree of threat, mainly, due to habitat destruction and selective extraction (Vásquez, 2000). The integration of activities focuses in conservation of these resources within native areas and the development of countryside populations is one of the main challenges to prevent the loss of biodiversity. In this sense *in vitro* culture techniques provide an alternative for sustainable management of this kind of natural resources. Through these, it's possible to obtain high amounts of plants for trade purposes, diminishing the pressure on the wild populations.

The project "Estudio del potencial de aprovechamiento sostenible de epifitas en el Parque Nacional y Área Natural de Manejo Integrado Cotapata (PN-ANMI Cotapata)" supported by Fondo Flamenco para el Bosque Tropical, has been developing diverse methodologies for the micropropagation of native orchids from the Bolivian Yungas. The plants obtained *in vitro* will be taken to a communal greenhouse located in "El Chairo" town for their acclimatization. The obtained income will be used to improve economy of the population located in PN-ANMI Cotapata. The presented data constitutes the preliminary results of a 3 years research.

Objectives

- Micropropagation of native orchid species from PN-ANMI Cotapata
- Evaluation of *in vitro* germinative response of 10 orchid species
- Comparison of the species' response under two germination treatments (media culture)

Methodology

Seeds were disinfected in 0.5% sodium hypochlorite (commercial solution) with two drops of a tensoactive agent during 18 minutes. The media culture used were MS (Murashige & Skoog 1962) and KC (Knudson C 1946) supplemented with 15% coconut milk, distributed in culture tubes (160 x 10mm.). This treatment was standarizated after preliminary test based on the protocol described by Villegas (2003). The evaluation of the *in vitro* germination was made six weeks after the introduction, quantifying the proportion of seeds that have reached any of the different germination stages based on the Pierik's germination criteria (1987) modified by Villegas (2003). Once the germinative process evaluation was finished, the

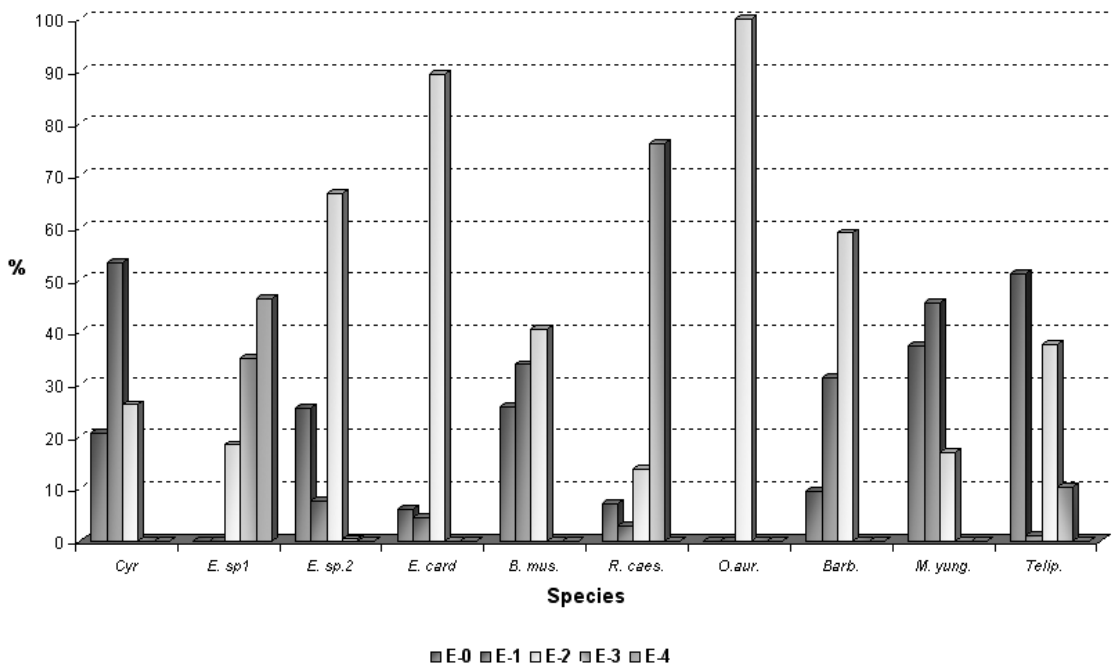

FIGURE 1. *In vitro* germinative process of 10 species of orchids in MS+15% L.C medium, after six weeks of evaluation. *Cyr. Cyrtochilum* sp., *E.* sp1. *Epidendrum* sp. 1, *E.* sp2. *Epidendrum* sp.2, *E. card. Epidendrum cardenasii, B. mus. Brachyonidium muscosum, R. case. Rusbyella caespitosa, O.aur. Odontoglossum aureum, Barb. Barbosella* sp., *M. yung. Masdevallia yungasensis, Telip. Telipogon* sp.

in vitro germinated species were transfered to MS medium without growth regulators for its further growth and development.

The studied species were: *Bracchionidium muscosum* Luer & Vásquez, *Epidendrum cardenasii* Hágsater, *Masdevallia yungasensis* Hashimoto, *Odontoglossum aureum* (Lindl.) Garay, *Rusbyella caespitosa* Rolfe, *Barbosella* sp. Schltr., *Cyrtochilum* sp. Khunt. *Epidendrum* L, *Epidendrum* L and *Telipogon* sp. Khunt. It is important to mention that for the last five cases, at this moment there is no taxonomic determination at species level, since two of them (*Cyrtochilum* sp. and *Telipogon* sp.) could be new species (R. Vásquez, pers. comm. 2006) while in the other cases the bloom time has been expected for their determination.

Results

After a six weeks evaluation, the most advanced stage of germination in both media culture was E-4 (protocorm with foliar primordial) seen only in *Epidendrum* (*E.* sp.1); in this species, the highest rate of germination was registered in KC medium (76.1%) in comparison with MS medium (46.5%). For the stage E-3 (protocorm), a favorable response was registered in KC medium too, since five of ten species studied reached it, while in MS medium only three species reached stage E-3. On the other hand, the species *Cyrtochilum* sp., *Bracchionidium muscosum, Odontoglossum aureum* and *Barbosella* sp. only reached stage E-2. In general, the highest rate of species in stage E-2 was observed in KC medium; however some species showed a more favorable response in MS medium (i.e. *Odontoglossum aureum* and *Barbosella* sp.). For stage E-0 (seeds that have not started the germinative process) *Masdevallia yungasensis* and *Telipogon* sp. showed highest rate in both media, showing a higher proportion in MS medium (Figures 1 and 2).

The results suggest that KC medium with 15% coconut milk is appropriate for the *in vitro* germination of the species studied in general, since in this medium the most advanced developmental stages (E-3 and E-4) were registered (Figure 3). Due to its chemical composition KC medium has been widely used for the *in vitro* orchids' germination (Pierik,

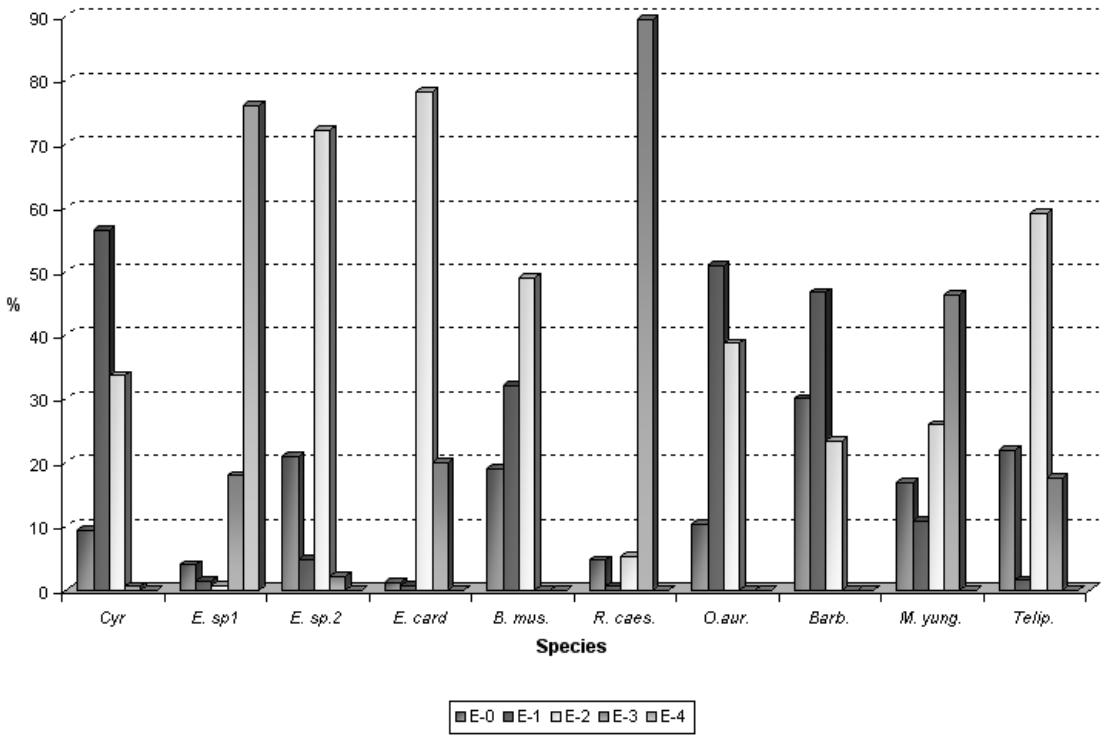

FIGURE 2. *In vitro* germinative process of 10 species of orchids in KC+15% L.C. medium, after six weeks of evaluation. *Cyr. Cyrtochilum* sp*., E.* sp1. *Epidendrum* sp.1*, E.* sp2. *Epidendrum* sp.*2, E. card. Epidendrum cardenasii, B. mus. Brachyonidium muscosum, R. case. Rusbyella caespitosa, O.aur. Odontoglossum aureum, Barb. Barbosella* sp*., M. yung. Masdevallia yungasensis, Telip. Telipogon* sp.

1987; Sánchez, 2006), however the present work showed that some species (*Odontoglossum aureum* and *Barbosella* sp.) have a more favorable response in MS medium according to the results obtained by Villegas (2003) in *Masdevallia chaparensis.*

At this time, the project "Estudio del potencial de aprovechamiento sostenible de epifitas en el PN-ANMI Cotapata" has at least 15 species in germination (without including those reported in this work), 30 in elongation and differentiation, 15 in multiplication and 3 in rooting process. The last ones are ready for acclimatization.

Conclusions

Usually, the most advanced stages (E-3 and E-4) were registered in KC medium.

Epidendrum sp.1 was the species with the best *in vitro* germinative response, since its seeds reached the stage E-4 (protocorms with foliar primordia).

The species *Odontoglossum aureum and Barbosella* sp. showed a more favorable germinative response in MS medium, in contrast to the other species studied.

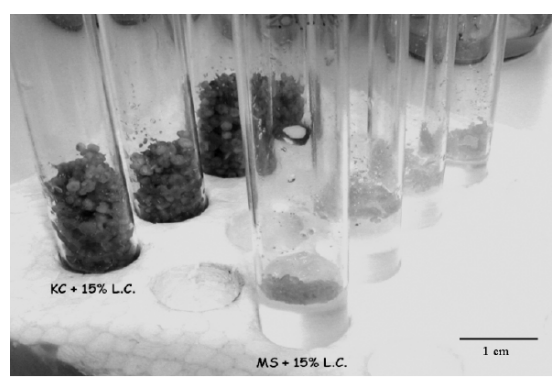

FIGURE 3. Effect of the medium composition on the germination of different orchids' species cultivated *in vitro*. MS+15% L.C: Murashige & Skoog (1962) medium supplemented with 15% of coconut water. KC+15% L.C.: Knudson C (1947) medium supplemented with 15% of coconut water.

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