

TRABAJOS ORIGINALES

Effects of photoperiod, plant growth regulators and culture media on *in vitro* growth of seedlings of *Cyrtorchilum loxense* (Lindl.) Kraenzl. an endemic and endangered orchid from Ecuador

Efectos del fotoperíodo, reguladores de crecimiento vegetal y medio de cultivo en el crecimiento *in vitro* de plántulas de *Cyrtorchilum loxense* (Lindl.) Kraenzl. una orquídea endémica del Ecuador

Yadira González¹ y Augusta Cueva A.^{1*}

1 Departamento de Ciencias Naturales, Universidad Técnica Particular de Loja, San Cayetano Alto. P.O. Box 608-1101 Loja, Ecuador.

* Autor para correspondencia. Fax: +593 72584893; Casilla postal: 11-01-608

Email: Yadira Gonzalez: xygonzalez@utpl.edu.ec

Email: Augusta Cueva: acueva@utpl.edu.ec

Citación:

González Y. & A. Cueva. 2014. Effects of photoperiod, plant growth regulators and culture media on *in vitro* growth of seedlings of *Cyrtorchilum loxense* (Lindl.) Kraenzl. an endemic and endangered orchid from Ecuador. *Revista peruana de biología* 21(2): 189 - 192 (Octubre 2014). doi: <http://dx.doi.org/10.15381/rpb.v21i2.9826>

Fuentes de financiamiento:

El presente trabajo fue financiado por la Universidad Técnica Particular de Loja.

Información sobre los autores:

Los autores declaran:

A.C. y Y.G. diseñaron los experimentos, analizaron resultados y escribieron el artículo. Y. G. desarrolló los experimentos.

Presentado: 06/02/2014
Aceptado: 16/08/2014
Publicado online: 07/10/2014

Journal home page: <http://revistasinvestigacion.unmsm.edu.pe/index.php/rpb/index>

© Los autores. Este artículo es publicado por la Revista Peruana de Biología de la Facultad de Ciencias Biológicas, Universidad Nacional Mayor de San Marcos. Este es un artículo de acceso abierto, distribuido bajo los términos de la Licencia Creative Commons Atribución-NoComercial-CompartirIgual 4.0 Internacional. (<http://creativecommons.org/licenses/by-nc-sa/4.0/>), que permite el uso no comercial, distribución y reproducción en cualquier medio, siempre que la obra original sea debidamente citadas. Para uso comercial, por favor póngase en contacto con editor.revperubiol@gmail.com.

Introduction

Orchidaceae is the largest of all vascular plant families present in Ecuador with nearly 4000 species 40% of which are endemic (Jørgensen & León-Yáñez 1999). Approximately 116 species distributed from Mexico to Bolivia belong to the genus *Cyrtorchilum* and sixty-five of these are present in Ecuador (Dodson & Escobar 2005). *Cyrtorchilum loxense* (Lindl.) Kraenzl. is an endemic species distributed in the high Andean forest of Southern Ecuador, near to Podocarpus National Park (Loja Province) (Endara et al. 2000). At present, due to high rate of deforestation, forest fires and habitat loss, this species is critically endangered (Dodson & Escobar 2005).

Cueva and González (2009) have previously determined the best conditions for germination of *C. loxense*. This study describes the effects of other parameters such as photoperiod, plant growth regulators as well as culture media to obtain optimal *in vitro* growth of this endangered orchid.

Material and methods

Eight-month old plants (approximately 1.5 cm in height), obtained through *in vitro* germination (Cueva & González 2009) were used for the evaluation of culture media, photoperiod and plant growth regulators treatments.

Culture media.- Murashige and Skoog (1962) (MS) and Knudson C (1946) media were tested to evaluate the nutrient influence on growth, shooting and rooting.

Plant Growth Regulators treatments.- Effects of plant growth regulators on growth, shooting and rooting were observed in plants cultivated by 60 days on MS with three combinations of α -naphthalene acetic acid (NAA) and 6-benzylaminopurine (BAP) (2 – 0.5, 1 – 0.5, and 0.5 – 0.5 mgL⁻¹, respectively). All culture media were supplemented with 20 g⁻¹ sucrose; the pH was adjusted to 5.8 with 1N KOH or HCl before adding 7 g⁻¹ agar (Bacto™ Agar 214010) and autoclaving at 121 °C for 20 min.

Photoperiod.- To determine the influence of photoperiod on the growth, shoot and root formation of *C. loxense*, day lengths of 8, 16 and 24 h, provided by 40W cool white fluorescent lamps (57 μ mol m⁻² s⁻¹) were tested. Plants were cultivated on MS medium augmented with 2 mgL⁻¹ glycine; 100 mgL⁻¹

myo-inositol; 0.05 mgL⁻¹ nicotinic acid and 0.05 100 mgL⁻¹ pyridoxine; during 60 days, and they were incubated in growth chambers at 22 \pm 1 °C.

Ten replicas (with ten plants per flask) per PGR and culture media treatment and five replicas for photoperiod were cultured. The growth (growth from the initial plant size, in cm), percentage of individuals forming new lateral shoots, shoot number, percentage of individuals forming roots and root number were register in seedling of *C. loxense* in 30 days intervals. All data were submitted of two-way variance analysis. Culture media data were submitted to Student's t test, with an alpha value of 0.05. Bonferroni's test was used to detect differences ($p \leq 0.05$) among plant growth regulators and photoperiod treatments.

Results and discussion

Culture media.- No significant differences in growth of *Cyrtorchilum loxense* were observed using the culture medium MS and Knudson C (Table 1). These results are in contrast with those recorded for other orchid species, like *Catasetum fimbriatum* and *Cyrtopodium paranaensis*, in which MS medium stimulated a better growth with respect to Knudson C medium after six months of culture (Rego Oliveira & Tadeo de Faria 2005). Since sucrose is a crucial component of culture medium, especially so for orchids (Pierik 1990), the same sucrose concentration in MS and Knudson C (2%), might explain the growth results.

The highest percentage of shoot formation after 60 days of culture (23%) was obtained using Knudson C medium as compared with 7% of shoot formation on MS (Figs. 1a, b). Shoot number was similar in MS and Knudson C. The highest percentage of root formation (82%) and the highest number of roots per explants (1.07) were obtained on Knudson C medium. In this medium, roots were longer and thicker than those formed on MS (Figs 2a, b). Contrarily, in *C. fimbriatum* y *C. paranaensis* these two culture media did not induce significant differences on root number and structure. It seems that the development in *C. loxense* could be stimulated by the presence of some nutrients available in Knudson C medium (George 1993b), a hypothesis that is in agreement with results reported for *Epidendrum* and *Laeliocattleya* (Churchill et al. 1972).

Plant growth regulators treatments.- None of the plant growth regulators evaluated caused significant differences in

Table 1. Effect of culture media Murashige and Skoog (MS). and Knudson C (KC) on growth, shooting and rooting of *Cyrtorchilum loxense*.

| Time | Morphogenic Responses | Culture Media | | P-value |
|---------|-----------------------|-----------------|-----------------|--------------|
| | | MS | KC | |
| 30 days | Growth (cm.) | 0.38 \pm 0.02 | 0.41 \pm 0.04 | 0.39 |
| | Shoot formation (%) | 0 | 7 \pm 3 | |
| | Shoot number | 0 | 0.19 \pm 0.14 | |
| | Root formation (%) | 27 \pm 4 | 39 \pm 5 | 0.64 |
| | Root number | 0.27 \pm 0.04 | 0.41 \pm 0.06 | 0.44 |
| 60 days | Growth (cm.) | 0.85 \pm 0.04 | 0.77 \pm 0.05 | 0.26 |
| | Shoot formation (%) | 7 \pm 2.1 | 23 \pm 4.4 | 0.007 |
| | Shoot number | 0.27 \pm 0.1 | 0.85 \pm 0.4 | 0.23 |
| | Root formation (%) | 63 \pm 4.9 | 82 \pm 4.8 | 0.007 |
| | Root number | 0.79 \pm 0.06 | 1.07 \pm 0.06 | 0.008 |

Data are present as mean \pm standard error. Data were compared with two-sided Student's t test with an alpha value = 0.05 and equal variance assumed

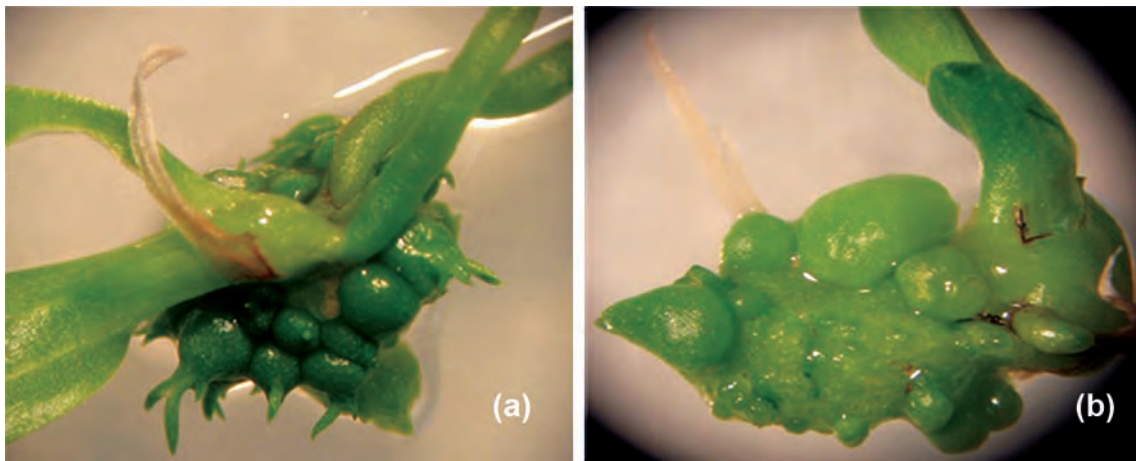


Figure 1. Protocorm like body formation of *Cyrtochilum loxense* at 60 days. (a) Kundson C medium. (b) MS medium.

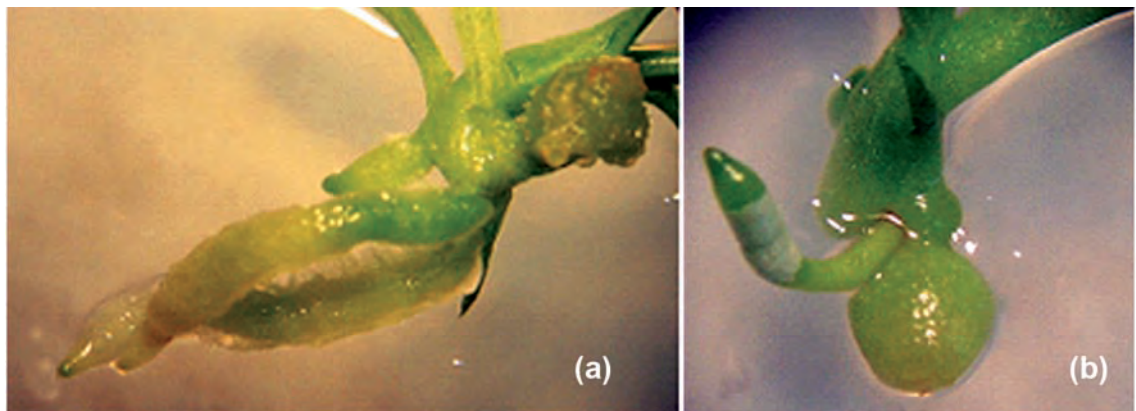


Figure 2. Rooting of *Cyrtochilum loxense* plants at 60 days: (a) Knudson C; (b) MS (1962) media.

growth and shoot formation. After 60 days, plant growth ranged between 0.75 – 0.86 cm (Table 2). The highest percentage of shoots (14%) and number of shoots per explants (0.57) were obtained on medium with 0.5 mg⁻¹L BAP and 1 mg⁻¹L of NAA (Table 2). The only statistical difference was observed on percentage of root formation (63%) and number of roots per explant (0.79) both on media without hormones. Krapiec et al. (2003) found the highest shoot induction in *Cattleya walkeri-ana*, on medium with the same plant growth regulators. Thus these results in the case of *C. loxense* could be attributed to the endogenous hormone concentration, since there are species for which external addition of plant growth regulators is not required (George 1993a).

Photoperiod.- To evaluate the effect of the photoperiod on growth, we inoculated explants in MS medium alone and added

with PGRs. The growth responses observed on MS without hormones were higher than those on media with plant growth regulators (Table 3). At day 45 the highest growth response was achieved under photoperiod 16/8 h. It is well known how light affects plant development, especially in *in vitro* culture conditions when plants are exposed to continuous light, a condition that can produce significant growth (Clouse 2001; Kim et al. 2002, Nemhauser & Chory 2002), for *C. loxense* at 60 days all plant growth regulators combinations present the highest growth under photoperiod 24/0 h (Table 3, Fig. 3). These results are similar to the results observed for *Psycmorchis pusilla*, for which the highest growth rate was observed with a photoperiod of 22/2 h (Vaz et al. 2004).

Summarizing we can conclude that Knudson C is a good medium for growing *C. loxense in vitro*, moreover it stimulates

Table 2. Effect of plant growth regulators on in vitro growth, shooting and rooting of *Cyrtochilum loxense* after 60 days.

| Plant Growth Regulators mgL ⁻¹ | | Growth (cm) | Shooting | | Rooting (p < 0.0001) | |
|---|-----|-------------|---------------------------------|----------------------------------|----------------------|---------------------|
| BAP | NAA | | Shoot formation (%) (p < 0.074) | Shoot number/explant (p < 0.412) | Root formation (%) | Root number/explant |
| 0.5 | 2 | 0.796a | 4 | 0.37a | 16b | 0.17b |
| 0.5 | 1 | 0.751a | 14 | 0.57a | 18b | 0.2b |
| 0.5 | 0,5 | 0.860a | 9 | 0.21a | 27b | 0.29b |
| 0 | 0 | 0.847a | 7 | 0.27a | 63a | 0.79a |

Growth, shoot number, rooting percentage and root number were analyzed by Duncan test; the data within the same column following by the same letter are not significantly different. Shoot formation data were analyzed using the Kruskal Wallis test.

Table 3. Effect of plant growth regulators and photoperiod on *in vitro* growth in *Cyrtorchilum loxense*.

| Plant Growth Regulators mg ⁻¹ L | | Photoperiod Light/Dark | Growth (cm) | |
|--|-----|------------------------|-------------|---------|
| BAP | ANA | | 45 days | 60 days |
| 0.5 | 2 | 24/0 | 0.6 cd | 0.7 |
| 0.5 | 2 | 16/8 | 0.5 d | 0.6 |
| 0.5 | 2 | 8/16 | 0.6 cd | 0.6 |
| 0.5 | 1 | 24/0 | 0.6 bcd | 0.8 |
| 0.5 | 1 | 16/8 | 0.6 cd | 0.6 |
| 0.5 | 1 | 8/16 | 0.6 cd | 0.7 |
| 0.5 | 0.5 | 24/0 | 0.7 abcd | 0.8 |
| 0.5 | 0.5 | 16/8 | 0.6cd | 0.7 |
| 0.5 | 0.5 | 8/16 | 0.5 cd | 0.6 |
| 0 | 0 | 24/0 | 0.9 ab | 1.2 |
| 0 | 0 | 16/8 | 1 a | 1.2 |
| 0 | 0 | 8/16 | 0.8 abcd | 1 |

Means in the same column followed by the same letter are not significant different (Duncan $p < 0.0001$).

good shooting and rooting after 60 days. However given that these three experiments were performed simultaneously, we evaluated only MS medium to verify the influence of PGRs and photoperiod. The use of 0.5 mgL⁻¹ of BAP and 1 mgL⁻¹ of NAA on MS medium improve shooting, on the other hand the best medium for rooting was MS medium without hormones. The best photoperiod for growing was 16/8 h, when MS without PGRs was used.

Literature cited

- Churchill M., E. Ball & J. Arditti. 1973. Tissue culture of Orchids. I: Methods for leaf tips. *New Phytologist* 72:161-166. <http://dx.doi.org/10.1111/j.1469-8137.1973.tb02021.x>
- Clouse S. 2001. Integration of light and brassinosteroid signals in etiolated seedling growth. *Trends Plant Science* 6:443-445. [http://dx.doi.org/10.1016/S1360-1385\(01\)02102-1](http://dx.doi.org/10.1016/S1360-1385(01)02102-1)
- Cueva A. & Y. González. 2009. *In vitro* germination and somatic embryogenesis induction in *Cyrtorchilum loxense*, an endemic, vulnerable orchid from Ecuador. In Pridgeon and Suárez (Eds.) *Proceedings of the Second Scientific Conference on Andean Orchids*. UTPL Publishers. Pp. 56-62.
- Dodson C. & R. Escobar. 2005. *Native Ecuadorian Orchids*. Colina Editorial. Medellín. Colombia: Pp. 665
- Endara L., C. Dodson, L. Jost & A. Tye A. 2000. Orchidaceae. In: Valencia R., Pitman N., León-Yáñez S., Jörgensen P. (Eds.) *Libro Rojo de las Plantas Endémicas del Ecuador*. Publicaciones del Herbario de la Pontificia Universidad Católica de Quito – Ecuador. Pp. 257-372
- George E. 1993a. *Plant Propagation by tissue culture Part 1: The Technology*. Second edition. Exegetics limited. Pp. 574.
- George E. 1993b. *Plant Propagation by tissue culture Part 2: In Practice*. Second edition. Exegetics limited. Pp. 1361.
- Jörgensen P. & S. León-Yáñez. 1999. *Catálogo de plantas vasculares del Ecuador*. Missouri Botanical Garden Pres, Editors. Quito-Ecuador. Pp. 1182.

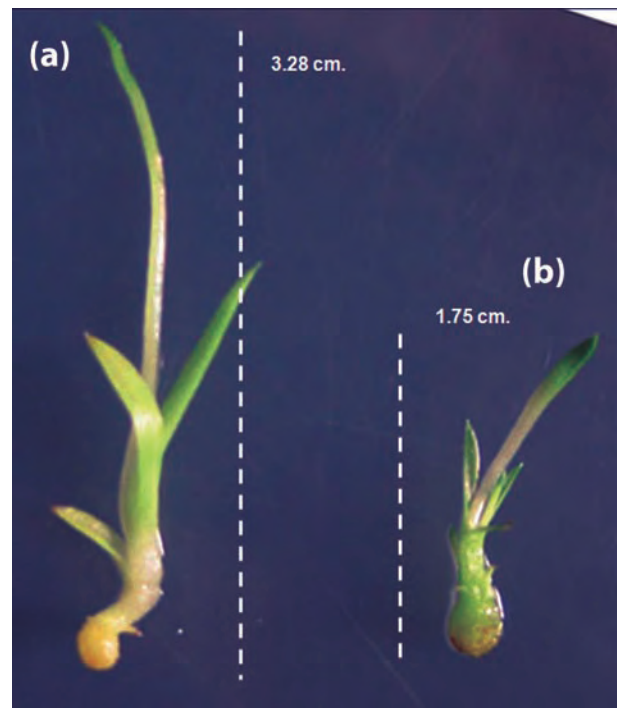


Figure 3. High of *Cyrtorchilum loxense* growing in MS with PGR at 60 days. (a) 24/0 light/darkness; (b) 8/16 light/darkness.

- Kim T., B. Kim & A.G. von Arnim. 2002. Repressors of photomorphogenesis. *International Review of Cytology* 220: 185 – 223. [http://dx.doi.org/10.1016/S0074-7696\(02\)20006-6](http://dx.doi.org/10.1016/S0074-7696(02)20006-6)
- Knudson L. 1946. A new nutrient solution for the germination of orchid seeds. *Botanical Gazette* 73:1-25. <http://dx.doi.org/10.1086/332956>
- Krapiec P., M. Milaneze & M. Pires da Silva. 2003. Effects of different combinations of growth regulators for bud induction from seedlings of *Cattleya walkeri* Gardner (Orchidaceae). *Acta Scientiarum: Biological Sciences*. 25: 179 - 182.
- Murashige T. & F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15:473-497. <http://dx.doi.org/10.1111/j.1399-3054.1962.tb08052.x>
- Nemhauser J. & J. Chory. 2002. Photomorphogenesis. In: Somerville C., Meyerowitz E. (Eds.) *The Arabidopsis Book*. American Society of Plant Biologist.
- Pierik R. 1990. *Cultivo in vitro de las Plantas Superiores*. Ediciones Mundi-Prensa. Madrid – España: 326.
- Rego-Oliveira L. & R. Tadeo de Faria. 2005. *In vitro* propagation of Brazilian orchids using traditional culture media and commercial fertilizers formulations. *Acta Scientiarum. Agronomy*, 27: 1 – 7.
- Vaz A., R. Figueiredo & G. Kerbauy. 2004. Photoperiod and temperature effects on *in vitro* growth and flowering of *P. pusilla*, an epiphytic orchid. *Plant Physiology and Biochemistry*, 42: 411 – 415. doi: <http://dx.doi.org/10.1016/j.plaphy.2004.03.008>