OPTIMIZATION OF BASAL CULTURE MEDIUM AND MICROPROPAGATION IN VITRO OF MEDICINAL PLANT *Castilleja tenuiflora* Benth IN A TEMPORARY IMMERSION BIOREACTOR.

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Key words: Micropropagation, *C. tenuiflora*, Temporary Immersion Bioreactors

Introduction. Biotechnological tools as plant cells culture *in vitro* have allowed micropropagation of plants with quality to produce plants under controlled conditions. *C. tenuiflora* (Orobanchaceae before Scrophulariaceae) or cancer herb accumulates secondary metabolites such as flavonoids, iridoids and phenolic compounds. These compounds have demonstrated biological activity, including anti-inflammatory, antioxidant, and cytotoxic activities, which may be related to the traditional uses of this plant. The aim of this work was to establish shoots culture of *C. tenuiflora* in a temporary immersion bioreactor.

Methods. Optimal culture medium (MCmod) for C. tenuiflora was defined using a Taguchi orthogonal design L9 considering the following factors: A) nitrate:ammonium ratio; B) sucrose concentration; C) growth regulators: thidiazuron and D) spermine. Each factor was studied at three levels. Subsequently, the influence of the time and dip frequency on the shoots multiplication (SM) and characteristics of shoots were assessed using basal medium without growth regulators B5 (RII). MCmod was tested in a temporary immersion bioreactor. Ten shoots of 1 cm and 21 days of age were inoculated in each experimental condition. For all experiments the culture conditions were 25 ± 2 ° C, photoperiod of 16 h light / 8 h dark.

Results. Optimal culture medium was of the combination of nitrate:ammonium 24:1, sucrose 45 g/L and spermine 5 µM without thidiazuron. The time and dip frecuency of reactor of 5 min of immersion every 24 had a significant effect ($p<0.05$) in combination with MCmod (Table 1). Ten shoots/explant was obtained and measured of 3-4 cm. All the shoots obtained by temporary immersion culture formed roots (Figure 1C,D) and showed a 100% survival when transferred to solid substrate.

Table 1. Growth characteristics shoots of *C. tenuiflora* culture in temporary immersion at 5 min every 24 h with the culture medium changed (MCmod) after 21 days of culture.

Culture Medium	SM	Height (cm)	Fresh Biomass g/Bioreactor	Dry Biomass g/Bioreactor	FR (%)
B5 basal	6±0.5**b**	3.88±0.2**a**	7.68±0.5**a**	0.59±0.03**a**	100**a**
MC mod	10±1.0**a**	3.73±0.2**a**	9.0±0.5**a**	0.654±0.03**a**	100**a**

Means ± standard error (Height: n= 150; SM and biomass: n= 5; FR: n=50). Different letters in the same column indicate significant differences (α = 0.05).

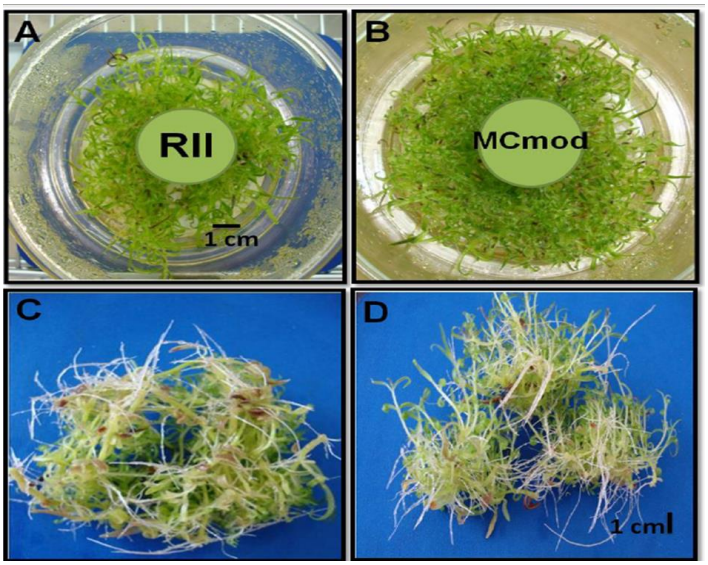

Figure 1. Growth characteristics shoots of *C. tenuiflora* in culture in temporary immersion, with 5 minutes immersion every 24 hours for 21 days of culture.

Conclusions. The temporary immersion culture of *C. tenuiflora*, with an immersion time of 5 minutes every 24 hours with the culture medium MCmod (nitrate: ammonium ratio 24:1, sucrose concentration 45 g / L and 5 µM spermine) afforded SM 9-11 shoots per explant capable of forming root. These shoots had 100% survival when transferred to solid substrate. Culture in temporary immersion represents a reliable and efficient methodology for *in vitro* micropropagation of *C. tenuiflora.*

Acknowledgements. This work was supported by SIP-IPN (20080101, 20090311y 20100308). R.V. also acknowledges the scholarship from COFAA and CONACYT.

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