Preliminary identification of alkaloids in *in vitro* cultured *Rhodophiala bifida* (Amaryllidaceae) plants

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Introduction

The plants of the Amaryllidaceae family are well known for their ornamental varieties and also for the production of a unique group of isoquinoline alkaloids. The Amaryllidaceae-type alkaloids have shown antiviral, antitumor and anticholinesterase activities. Montanine[1], is an alkaloid isolated from *Rhodophiala bifida* plants which has antioxidant, anti-inflammatory and antimicrobial properties. To date, there is no report on plant regeneration from this species. Plant biotechnology is a useful method which aims to obtain these compounds and to produce plants capable to yield them. Based on the foregoing, due to the importance of medicinal alkaloids produced by these plants and the use of tissue culture as an alternative production source of such phytochemicals, these studies are justified to undertake the development of *in vitro* techniques that provides future opportunities for the Amaryllidaceae alkaloids, like montanine and the production in this type of culture. The goal of this study was to propagate *Rhodophiala bifida*, an Amaryllidaceae, plant species with montanine content, by organogenesis.

Results and Discussion

The best organogenesis results were obtained after 2 months cultured in MS medium with modifications[2] + 0,1 mg L^{-1} NAA+ BA 0,5 mg L^{-1} (Table 1), where 36% of the explants produced buds. These conditions generated small bulbous plantlets, after 40 days in culture.

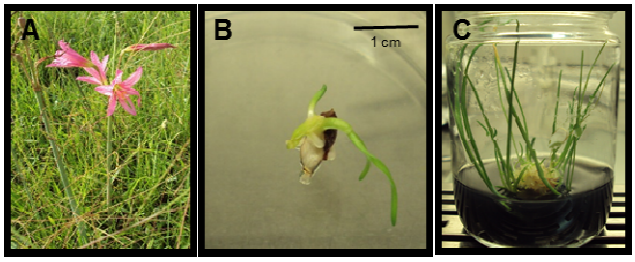

Figure 1. *Rhodophiala bífida* plants, cultivated *in nature* (A) and obtained by direct organogenesis, through bulbar explants (B), *R. bifida* plants *in vitro*.

The grown R. *bifida in vitro* plants (Figure 1C), after 60 days of growth, were analyzed for the production of montanine, remarkable alkaloid of this type, when *in nature*. Through HPLC analysis, we can determine the presence of the retaining point and the characteristic UV spectra of this molecule (Figura 2).

Table 1. Effects of different media in the percentage of shoot formation in *Rhodophiala bifida* explants, on different days of treatment.

Medium	20 days	40 days	60 days
I	0a	4b	8bc
II	8a	12b	20ab
III	0a	0b	4bc
IV	0a	0b	0c
V	0a	0b	0c
VI	0a	3,3b	3,3bc
VII	6,3a	31,25a	36,25a
VIII	6,7a	15ab	15bc
IX	0a	0b	6,7bc
X	0a	0b	0c

Values in the same column, with same letters, are not statiscally different according to Tukey ($p< 0,05$).

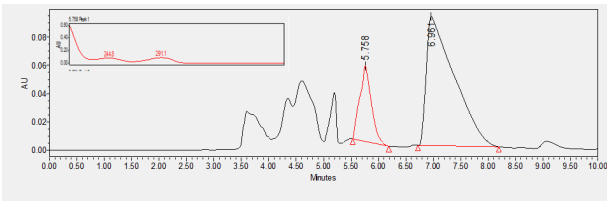

Figure 2. Chromatogram and UV absorption spectrum of *Rhodophiala bifida* plants cultured in vitro in MS + 0,1 mg L^{-1} NAA+ 0,5 mg L^{-1} BA, after 60 days of treatment. Montanine retention time in 5.758 minutes.

Conclusions

We indicate an attempt to produce montanine alkaloid, for the first time, in *Rhodophiala bifida in vitro* plant cultures.

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