SUSTAINABLE AND INTEGRAL EXPLOITATION OF AGAVE

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CONACYT

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MICROPROPAGATION OF *Agave victoriae-reginae* (T. MOORE) IN A TEMPORARY IMMERSION SYSTEM

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ABSTRACT

Agave victoriae-reginae T. Moore is an endemic species from Mexico which due to its high ornamental value has been submitted to an extensive illegal collection for commercial trade. Currently, it is listed as extinction endangered species by the Mexican government. In the present study the *in vitro* multiplication of *A. victoriae-reginae* was attempted for the first time by using a new temporary immersion system. Murashige and Skoog´s medium supplemented with Kinetin (Kn), 6-benzylaminopurine (BA), and Thidiazuron (TDZ) was tested at various concentrations, being the immersion frequency of one minute a day. Results showed that the best proliferation response was reached in a culture medium supplemented with 0.53 mg/L IBA and 0.1 mg/L BA, resulting in 7 shoots per initial explant after nine weeks of culture. This micropropagation system constitutes a promissory alternative to repopulate and conserve the *A. victoriae-reginae* natural populations for present and future.

Keywords: *Agave victoriae-reginae, conservation, endemic, in vitro, temporary immersion, micropropagation*

INTRODUCTION

Agave victoriae-reginae is an endemic plant from Mexico, which is catalogued as an extinction threatened species according to the NOM—059—SEMARNAT—2010 and the Appendix II by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Those plants had been subjected to intensive exploitation due its great value, mainly as exceptional ornamental plants. As result, their wild populations have been drastically reduced due to over collection and severe perturbation of their native habitat. All these facts make *A. victoriae-reginae* an important plant to be conserved by all means, including the *in vitro* culture.

Previous reports have established *in vitro* regeneration protocols for *A. victoriae-reginae* propagation whether by indirect somatic embryogenesis (Rodriguez-Garay et al., 1996) or

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by direct organogenesis design (Martínez-Palacios et al., 2003; Ramírez-Malagón et al., 2008). These authors cultured their *A. victoriae-reginae* plants on a semi-solid substrate including a gelling agent, e.g. agar, which is one of the most expensive ingredients in those culture media. Handling of that vegetal material and its periodic transfer to new media is time consuming and causes contamination and tissue damage (Weathers and Giles, 1988).

To overcome these difficulties associated with semisolid media; the propagation system based in temporary immersion provides an interesting approach. This system consists in the immersion of plant tissue during specific time periods in the culture medium (Etienne and Berthouly, 2002). The aim of this research was to develop an efficient protocol for the *in vitro* propagation of *A. victoriae-reginae* plant in a new temporal immersion system.

METHODOLOGY

Plant material

Leaves (5–8 cm length) of *Agave victoriae-reginae* plants, previously *in vitro*-grown by Dr. Rafael Ramírez Malagón on MS medium containing agar (0.8%), were inoculated into MS liquid medium (Murashige and Skoog, 1962).

Shoot induction

Explants were inoculated into translucent and autoclavable glass bottles (15x15x50 mm), containing 50 ml MS liquid medium, which operates on the principle of temporary immersion. In this system, plants are placed on a plastic net that separates the vegetal material from the liquid media. For shoot induction twelve treatments were evaluated. The basal MS medium was supplemented with different levels of cytokinins; such as Kinetin (Kn), 6-benzylaminopurine (BA), and Thidiazuron (TDZ), and the auxin indole butyric acid (IBA), according to Ramírez-Malagón et al. (2008) and Martínez-Palacios et al. (2003) (Table 1). The media pH was adjusted to 5.6-5.8 in all cases.

Table 1. Description of plant hormones used for the shoot induction of *Agave victoria-reginae* in a temporary immersion system.

Treatments	Plant hormones (mg L^{-1})			
	IBA	BA	Kn	TDZ
T1	0.53	0.1	-	-
T2	0.53	0.5	-	-
T3	0.53	1	-	-
T4	-	0.1	-	-
T5	-	0.5	-	-
T6	-	1	-	-
T7	-	-	1	-
T8	-	-	3	-
T9	-	-	5	-
T10	-	-	-	0.1
T11	-	-	-	0.2
T12	-	-	-	0.3

One shoot was added per vessel and every experiment was repeated four times. Bottles were randomly placed in the growth chamber and maintained at 25°C under a 16-h photoperiod provided by cool white fluorescent lamps (23-26 μmol m^{-2} seg^{-1}). Temporary immersion cultures were established with immersion of explants for 1 min every 24 h. Variance analysis was conducted to evaluate the results, and Tukey´s test was used to separate the data means.

RESULTS AND DISCUSION

New adventitious shoots emerged from initial explant after nine weeks of culture for all treatments. Analysis of variance indicated that medium composition significantly affected the shoot number developed per explant.

The treatment containing 0.53 mg/L IBA and 0.1 mg/L BA (T1) showed the best multiplication rates, with around of seven shoots per explant. The rest of treatments not exceeded three shoots per explant, being less efficient than treatments T10 and T11 (Figure 1).

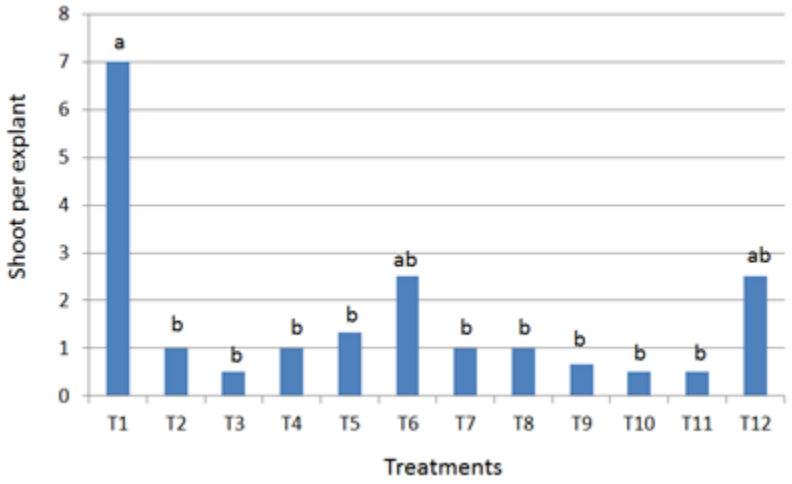

Figure. 1. Plant hormone effects on *Agave victoria-reginae* shoot induction growning in a temporary immersion system. T1 to T12 correspond to the twelve treatments evaluated using MS medium supplemented with different plant hormones combinations. The bars with different letters indicate significant differences between treatments at $p<0.05$ according to Turkey's test.

After nine weeks of culture, the agave plants remained with vigorous appearance and without any sign of hyperhydricity. The necrotic tissue presence was lower than 5% for all evaluated treatments (Figure 2).

Figure. 2. *Agave victoriae-reginae* plants after nine weeks grown on temporary immersion system.

Up to our knowledge, there are a few reports about the *in vitro* regeneration of *A. victoriae-reginae*. In those previous studies, the addition of BA favored the shoot proliferation even when only 1.1-2.2 axillary shoots were regenerated from *A. victoria.reginae* stems (Martínez-Palacios et al., 2003). Likewise, only 5.5 axillary shoots per explant of *A. victoriae-reginae*, using 2.46 µM IBA and 2.22 µM BA after 60 days of culture in semisolid medium, were induced (Ramirez-Malagón et al. 2008). Nonetheless, no reports were found about the *A. victoria reginae* shoot multiplication using a liquid culture system. In this regard slightly greater plant yields were obtained in the present study. In general, liquid culture systems can provide much more uniform conditions into the plants containers, due to the occurrence of a close contact and uniform entrance of nutrients and plant growth regulators to the explants. Also, the culture atmosphere is renewing in each immersion resulting in better growth rates (Etienne and Berthouly, 2003). In addition, the liquid system is useful to increase the scale of production and also enables its future automation reducing the production costs per plant. This fact converts the temporary immersion systems in a relevant tool to accelerate the conservation programs of extinction threated species.

CONCLUSIONS

This study demonstrated that it was possible to regenerate, in a practical way, *A. victoriae-reginae* vigorous shoots by using a temporal immersion system. The MS culture media supplemented with 0.53 mg/L IBA and 0.1 mg/L BA (T1) was found to be the most effective for inducing vigorous shoots of *A. victoriae-reginae*, obtaining 7 shoots per explant and suggesting the possibility of large-scale multiplication of this important endangered agave species.

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