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Induction of somatic embryogenesis in *Heliconia chartacea* Lane ex Barreiros cv. Sexy pink and cv. Sexy scarlet from sections of ovaries[1]

ABSTRACT

The aim of the present paper was to establish protocols for the induction of embryogenic callus in *Heliconia chartacea* Lane ex Barreiros cv. Sexy Pink and Sexy Scarlet from ovaries sections. The experiment tested combinations between IAA (0; 1 and 2 mg L^{-1}) and 2.4-D (0; 5; 10; 15 and 20 mg L^{-1}) in the cultivars Sexy Pink and Sexy Scarlet. The cultivar Sexy Pink presented a higher frequency of callus development as compared to Sexy Scarlet. In the treatments A1D10 (1mg L^{-1} of IAA and 10mg L^{-1} of 2.4-D) and A2D5 (2 mg L^{-1} of IAA and 5mg L^{-1} of 2.4-D), the callus frequency of formation of the cultivar Sexy Pink corresponded to 100% of the inoculated explants, showing that the IAA associated with the 2.4-D induced a satisfactory callus formation response in the studied cultivar.

Key words: callogenesis, growth regulator, histology, scanning electron microscopy

Indução de embriogênese somática em *Heliconia chartacea* Lane ex Barreiros cv. Sexy pink e cv. Sexy scarlet provenientes de secções de ovários

RESUMO

O presente trabalho teve como objetivo estabelecer protocolo para indução de calos embriogênicos em *Heliconia chartacea* Lane ex Barreiros cv. Sexy Pink and Sexy Scarlet a partir de secções de ovários. O experimento avaliou combinações entre IAA (0; 1 e 2 mg L^{-1}) e 2,4-D (0; 5; 10; 15 e 20 mg L^{-1}) nas cultivares Sexy Pink e Sexy Scarlet. A cultivar Sexy Pink apresentou maior frequência quanto ao desenvovimento de calos comparada com a cultivar Sexy Scarlet. Nos tratamentos A1D10 (1 mg L^{-1} de IAA e 10 mg L^{-1} de 2,4-D) e A2D5 (2mg L^{-1} de IAA e 5mg L^{-1} de 2,4-D), a frequência de formação de calos na cultivar Sexy Pink correspondeu a 100% dos explantes inoculados, demonstrando que o IAA associado ao 2,4-D induziu satisfatoriamente a formação de calos na cultivar estudada.

Palavras-chave: calogênese, reguladores de crescimento, histologia, microscopia eletrônica de varredura

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INTRODUCTION

Ornamental tropical plants have attracted the flower market due to beauty, exoticism and post-harvest inflorescences durability. In Brazil, the production of heliconia has grown significantly recently, especially in the Northeastern region. In this region, small and medium size producers are taking advantage of the favorable marketing conditions associated with the beautiful inflorescences that form as a result of ideal environment conditions for heliconia production.

The helicons possess a subterraneous rhizome, commonly used for propagation from which new buds are developed (Castro, 1995). This system of vegetative propagation, in addition to produce a reduced number of plants offers serious risks of plague and disease dissemination. Nevertheless, the micropropagation of helicons via tip shoots faces problems of endophytic contamination, which limits the explant development, as registered by several authors (Nathan et al., 1992; Atehortua et al., 1999; Dias & Rodrigues, 2001). In face of this problem, the use of other types of explants associated with other micropropagation techniques becomes necessary. The micropropagation techniques can offer important tools to solve problems that limit the growth of this important ornamental culture. Among the propagation and breeding problems of this genus are the slow germination rates (around 3 months to 1 year) and the asexual propagation by rhizomes, which are underground structures, slow-growing and difficult to monitor (Atehortua, 1997).

Somatic embryogenesis is one of the micropropagation techniques that stand out for presenting some advantages. It makes possible the attainment of countless propagules from a reduced number of explants (Vasil & Vasil, 1986; Vasil, 1988; Jiménez, 2001; Maciel et al., 2003), offers opportunities for *in vitro* production of true-to-type plants by clonal propagation as well as regeneration of genetically modified plants by genetic transformation, somatic hybridisation and *in vitro* mutant induction and selection. Moreover, it is a useful tool in basic research on totipotency and on the fundamental processes of plant morphogenesis (Guerra et al., 1999; Jiménez, 2001; Gaj, 2004).

Thus, this work aimed at induction of somatic embryos in *H. chartacea* Lane ex Barreiros cv. Sexy Pink and cv. Sexy Scarlet.

MATERIAL AND METHODS

The ovaries extracted from inflorescences with four open and one closed bract, were used as explant. Initially, these explants were washed with pure commercial detergent and running water. Then they were immersed in alcohol at 70%, for one minute, followed by one immersion in calcium hypochlorite solution (3%); containing three drops of 20% tween, for 20 min; then a second immersion in calcium hypochlorite solution (1.5%) also containing three drops of 20% tween, for 15 min; and finally three washes with sterile distilled water to eliminate the excess of the disinfectant agents. After this process, the ovaries were cross-sectioned and the sections, measuring approximately 0.7 cm in diameter and 0.2 cm in thickness (2.5 x 15 cm) containing 10 mL of MS medium (Murashige & Skoog, 1962).

The experiment consisted of combinations among three levels of IAA (0; 1 and 2 mg L^{1}) and five levels of 2,4-D (0; 5; 10; 15 and 20 mg L^{-1}) in the cultivars Sexy Pink and Sexy Scarlet, in a 3x5x2 factorial scheme, with an experimental outline entirely randomized, with 10 replicates per treatment.

The basic MS medium was increased by 30 $g.L^{-1}$ of sucrose and 2 g L^{-1} of phytagel. The pH was adjusted to 5.8 before sterilization in autoclave at 121°C, for 20 minutes. The inoculated material was kept in the dark, at a temperature of $25^{\pm}2$ °C, for 90 days.

The evaluations were carried out at 30, 60 and 90 days after inoculation, based on visual observations and qualitative characteristics, with the aid of a stereomicroscope. The induction frequency (%) of callus was calculated by (number of explants formed callus/total number of explants X 100). A non-parametric statistical test was applied to the ICF, based on the distribution of Qui-square, using a Tukey test to be employed in the data contrasts transformed into arcsen $\sqrt{\%}$.

RESULTS AND DISCUSSION

The cultivar Sexy Pink presented a higher frequency of callus development as compared to Sexy Scarlet. In the treatments A1D10 and A2D5, the frequency of callus the cultivar Sexy Pink which corresponded to 100% the inoculated explants (Table 1), showing that the IAA associated with the 2.4-D induce a satisfactory the callus formation responses in the cultivate studied. Nathan et al. (1993) obtained 100% of callus development in a treatment with a higher rate of 2.4-D (17.68 $mg.L^{-1}$), using as initial explant segments of *H. psittacorum* shoots obtained *in vitro*.

One of the factors that explain the fact that the cultivar Sexy Scarlet has not responded to the applied treatments may be associated with the genotype (recalcitrant). Even though the cultivars are of the same species (*H. chartacea* cv. Sexy Pink and Sexy Scarlet), the genotype exerts an influence on the *in vitro* behavior, and this may be associated with the recalcitrance of particular monocotyledons (Liliopsids). Several researchers have observed this influence, which suggest that dominant genes control the development of embryogenic callus and the regeneration of plants in *in vitro* cultivation (Green & Rhodes, 1982; Tomes, 1985).

In a great number of processes, the control of the genic activity in the transition and translation is directly related to the responsive cells. These responsive cells are characterized by the presence of receptors that connect with the hormone and, subsequently, initiate the response in the cell. These receptors are generally of proteic origin (Guerra et al., 1999).

It was observed that most of the ovary sections of the cv. Sexy Scarlet used as explants did not show swelling during the 90 days of cultivation, except in the treatments A1D5 e A2D10, in which there appeared friable callus of whitish yellowish color, developing more in the central portion of the

Table 1. Frequency of callus formation from ovaries sections of the cultivars Sexy Pink and Sexy Scarlet of the species *H. chartacea* Lane ex Barreiros at 90 days, in mediums with different combinations of IAA and 2.4-D (mg L^{-1})

Tabela 1. *Freqüência de formação de calos de secções de ovários da cultivar Sexy Pink and Sexy Scarlet of the species H. chartacea Lane ex Barreiro aos 90 dias em meios com diferentes concentrações de AIA e 2,4 D (mg L^{-1})*

Treatments (mg L^{-1})	Frequency of formation callus (%)	
	Sexy Pink	Sexy Scarlet
0 AIA + 0 2.4-D (A0D0)	0 b	0 a
0 AIA + 5 2.4-D (A0D5)	80 a	0 a
0 AIA + 10 2.4-D (A0D10)	60 ab	0 a
0 AIA + 15 2.4-D (A0D15)	80 a	0 a
0 AIA + 20 2.4-D (A0D20)	20 ab	0 a
1 AIA + 0 2.4-D (A1D0)	40 ab	0 a
1 AIA + 5 2.4-D (A1D5)	60 ab	40 a
1 AIA + 10 2.4-D (A1D10)	100 a	0 a
1 AIA + 15 2.4-D (A1D15)	80 a	20 a
1 AIA + 20 2.4-D (A1D20)	80 a	20 a
2 AIA + 0 2.4-D (A2D0)	60 ab	0 a
2 AIA + 5 2.4-D (A2D5)	100 a	20 a
2 AIA + 10 2.4-D (A2D10)	80 a	40 a
2 AIA + 15 2.4-D (A2D15)	60 ab	20 a
2 AIA + 20 2.4-D (A2D20)	0 b	20 a

Averages followed by different letters between the lines inside each column, differ among themselves at the 5% level of probability by the Tukey test.
Médias seguidas de diferentes letras entre linhas em cada coluna, diferem entre si ao nível de 5% de probabilidade pelo teste de Tukey.

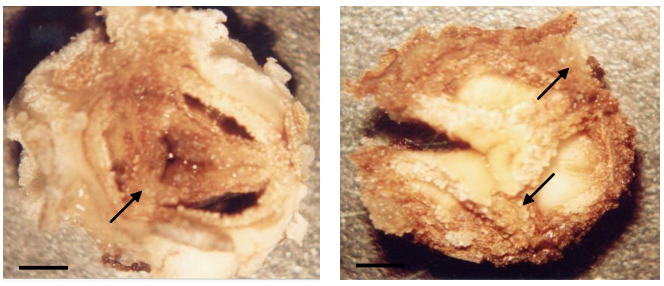

Figure 1. Callus derived from ovary sections (A) Callus obtained in cv. Sexy Scarlet in the treatment 1 mg L^{-1} of IAA + 5 mg L^{-1} of 2.4-D (A1D5), at 90 days of cultivation (arrow) (Bar = 1.25mm); (B) Callus obtained in cv. Sexy Pink in the treatment 1mg L^{-1} of IAA + 10mg L^{-1} of 2.4-D (A1D10), after 30 days of cultivation (arrows) (Bar = 1.25mm)

Figura 1. *Calos derivados de secções de ovários (1A) Calos obtidos da cv. Sexy Scarlet no tratamento com 1 mg L^{-1} de IAA + 5 mg L^{-1} de 2.4-D (A1D5), aos 90 dias de cultivo (seta) (Barra = 1,25 mm); (B) Calos obtidos da cv. Sexy Pink no tratamento com 1 mg L^{-1} de IAA + 10 mg L^{-1} de 2,4-D (A1D10), aos 30dias de cultivo (seta) (Barra = 1,25 mm)*

explant (Figure 1A). There was no development of somatic embryos from these callus. On the other hand, at 30 and 60 days of cultivation, most of the explants of cv. Sexy Pink did present swelling of the edges, which progressed into callus with friable characteristics, of cream to yellowish coloring (Figure 1B). But at the end of the 90 days, the presence of embryogenic structures; that is, the callus only presented a cellular mass, without the development of nodules that characterize the presence of somatic embryos. The cytochemical analysis of these callus did not show the presence of pro-embryogenic cells. This fact may be attributed to the high concentrations of auxins used in this essay, interfering with the development of somatic embryos, as observed in the treatment with 2 mg L^{-1} of IAA and 20 mg L^{-1} of 2.4-D (A2D20) (Table 1). In face of the results obtained a dependence on exogenous auxin for the induction of callus was verified, since in the control treatment (A0D0) there was no callus development, but it was also verified that high concentrations of this growth regulator also inhibit callogenesis.

LITERATURE CITED

Atehortua, L. Heliconias: A new challenge for the Colombian floricultural industry. Biotecnology Development Monitor, Colombia, n.31, p.20-21, 1997.

Atehortua, L.; Urrea, A. L.; Gil, U.; Mora, B.; Valencia, C.; Corrales, M.; Carmona, A.; Vallejo, A. Heliconia tissue culture. Colombia Bulletin of Heliconia Society International, Cali, v. 9, n.4, p.16-17, 1999.

Castro, C. E. F. Helicônia para exportação: Aspectos técnicos da produção. Brasília: Embrapa, (FRUPEX. Publicações Técnicas, 16). 1995. 44p.

Dias, M. A.; Rodrigues, P. H. V. Fontes de explantes e contaminantes isolados em cultivo *in vitro* de helicônia. Revista Brasileira de Horticultura Ornamental, Campinas, v.7, n.2, p.165-168, 2001.

Gaj, M.D. Factors influencing somatic embryogenesis induction and plant regeneration with particular reference to *Arabdopsis thaliana* (L.) Heynh. Plant Growth Regulation. v.43, p.27-47, 2004.

Green, C. E.; Rhodes, C. A. Plant regeneration in tissue culture of maize, In: Sheridan, W. F (ed.), Maize for biological research. Grand Forks: University Press, University of Dakota, 1982. p.367-372.

Guerra, M. P., Torres, A. C.; Teixeira, J. B. Embriogênese somática e sementes sintéticas. In: Torres, A. C.; Caldas, L. S.; Buso. J. A. (ed.). Cultura de tecidos e transformação genética de plantas. Brasília: EMBRAPA-SPI, 1999. p.533-568.

Jiménez, V. M. Regulation of *in vitro* somatic embryogenesis with emphasis on the role of endogenous hormones. Revista Brasileira de Fisiologia Vegetal, v.13**,** n.2. p.196-223. 2001.

Maciel, A. L. R.; Pasqual, M; Pereira, A. R.; Rezende, J. C.; Silva, A. B.; Dutra, L. F. Embriogênese somática indireta em explantes foliares de *Coffea arabica* L. cv. Oboatã. Revista Ciência e Agrotecnologia, Lavras, v.27, n.1, p.107-116, 2003.

Murashige, T.; Skoog, F. A revised medium for rapid growth and biossays with tobacco tissue culture. Physiologia Plantarum, v.15, p. 473-497, 1962.

Nathan, M. S.; Goh, C. J.; Kumar, P. P. *In vitro* propagation of *Heliconia psittacorum* by bud culture. Hortsience, v.27, p. 450-452, 1992.

Nathan, M. S.; Kumar, P. P.; Goh, C. High frequency plant regeneration in *Heliconia psittacorum* L.f. Plant Science, v.90, p.63-71, 1993.

Tomes, D. T. Cell culture, somatic embryogenesis and plant regeneration in maize, rice, sorghum and millets. In: Brigth, S.W.J.;Jones, M.G.K. (ed.). Cereal tissue and cell culture. Dordrecht, Boston: Martinus Nijhoff / Dr. W. Junk Publishers, 1985. p.175-205.

Vasil, I. Progress in the regeneration and genetic manipulation of cereal crops. Biotecnology, v.6, p.397-402, 1988.

Vasil, I. K.; Vasil, V. In cell culture and somatic cell genetics of plants. Orlando, Academic Press, 1986. 150 p.