CALLUS INDUCTION AND PLANT REGENERATION FROM *Anthurium andraeanum* Lindl. FRUITS

INDUÇÃO DE CALOS E REGENERAÇÃO DE PLANTAS A PARTIR DE FRUTOS DE *Anthurium andraeanum* Lindl.

MAURÍCIO REGINALDO ALVES DOS SANTOS[1], ANA LUIZA DE OLIVEIRA TIMBÓ[2], ANA CRISTINA PORTUGAL PINTO DE CARVALHO[3], JOÃO PAULO SARAIVA MORAIS[4]

[1]Doutor em Agronomia/Fitotecnia – Embrapa Agroindústria Tropical.
[2]Graduanda em Ciências Biológicas – Embrapa Agroindústria Tropical.
[3]Doutora em Ciências Biológicas/Genética – Embrapa Agroindústria Tropical.
[4]Mestrando em Bioquímica – Embrapa Agroindústria Tropical – Embrapa Rondônia – Caixa Postal 406 – 78900970 – Porto Velho, Rondônia – mauricioreginaldo@yahoo.com.br

Abbreviations: 2.4-D – 2.4-dichlorophenoxyacetic acid; BA - 6-benzyladenine; NAA - naphthaleneacetic acid

ABSTRACT

Anthurium andraeanum Lindl. plants are usually propagated by cuttings (suckers, stem sections and offshoots) or seedlings. This can take a very long time or result in high variability. The aim of this work was callus induction and plant regeneration from *A. andreanum* fruits (without seeds). MS basal medium was used without or supplemented with the following growth regulators: 2.22µ M BA; 8.88µM BA; 8.88 µM BA + 1.07 µM NAA; 9.05 µM 2.4-D; and 18.09 µM 2.4-D. After 1 month of culture the callus induction occurred. More effective media for callus induction were MS + 2.22 µ M BA and MS + 18.09 µM 2.4-D. The calli were transferred to a medium MS with 0,89 µ M BA. Two months after fruit inoculation, only one callus grew up and was chlorophylled. After 3 months occurred the regeneration of the plantlets. All the other calli browned, becoming necrotic. Rapid callus induction from *Anthurium andreanum* fruits is feasible, although future studies are required for callus induction and plant regeneration and multiplication.

Index terms: Micropropagation, organogenesis, ornamental plants, tissue culture, tropical plants, *Anthurium andraeanum.*

RESUMO

As plantas de *Anthurium andraeanum* Lindl. são geralmente propagadas vegetativamente (rizomas, touceiras ou brotos laterais) ou por sementes. Essas técnicas podem resultar em ciclos produtivos muito longos ou alta variabilidade das progênies. Objetivou-se com este trabalho induzir calos e regenerar plantas a partir de frutos (sem sementes) de plantas dessa espécie. Utilizou-se meio de cultura MS sem reguladores de crescimento e suplementado com 2,22 µM de BA; 8,88µ M de BA; 8,88µ M de BA + 1,07µM de ANA; 9,05µ M de 2,4-D; e 18,09µM de 2,4-D. Após um mês de cultivo, ocorreu a indução de calos. Os meios mais efetivos foram MS + 2,22µ M de BA e MS + 18,09µ M de 2,4-D. Os calos foram transferidos para meio MS + 0,89 µ M de BA. Aos dois meses de cultivo, somente um calo cresceu, apresentando-se clorofilado. Aos três meses de cultivo, obteve-se a regeneração de plântulas a partir desse calo. Todos os outros calos sofreram oxidação e necrose. A indução rápida de calos a partir de frutos de *Anthurium andraeanum* é possível, mas são necessários mais estudos sobre a indução de calos, regeneração e multiplicação de plantas.

Termos para indexação: Micropropagação, organogênese, plantas ornamentais, cultura de tecidos, plantas tropicais, *Anthurium andraeanum.*

INTRODUCTION

The plants of the species *Anthurium andreanum* Lindl. are well-known for the beauty of their leaves and for the size and colours of their inflorescences. They are one of the most sought after and used tropical flowers, due especially to the great longevity of the inflorescences (LAMAS, 2002).

The conventional propagation of this plant is usually done by cuttings (suckers, stem sections and offshoots) or seedlings. Both methods lead to excessively long production cycles. Due to cross fertilisation, seeds also possess the inconvenience of genetic heterogeneity and high variability in the progenies; the majority of seedlings do not have any commercial value (GEORGE, 1996).

As an alternative to massive propagation of this species, micropropagation by indirect organogenesis has been used, that is, the explant passes by the stage of callus

(Recebido em 28 de junho de 2004 e aprovado em 03 de novembro de 2004)

before plant regeneration (HAMIDAH et al., 1997). Its tissue culture was first reported by Pierik et al. (1974), further refined by many others (GEIER, 1990) and is now widely practised for commercial production.

For callus induction, foliar explants are commonly employed. However, this can take approximately three months, or requires the utilisation of zeatin (TOMBOLATO et al., 1998).

The aim of this study was to search for a less costly and faster way to induct callogenesis at *A. andreanum*, using fruits as explants, as well as regenerate plants from the calli.

MATERIALS AND METHODS

Mature fruits of *A. andreanum* cv. Cananéia were taken from spathes of plants from Tropical Flowers Germoplasm Bank of Embrapa Agroindústria Tropical, in Fortaleza, in the state of Ceará, Brazil. The spathes were washed with tap water and liquid soap (chlorhexidine digluconate 2%) and sprayed with an antibiotic solution (Sodium rifampicin 12.10µ M) in Petri dishes. After 2 hours, the spathes were disinfested with ethanol 70% for two minutes, calcium hypochlorite 2.5% for ten minutes and carbendazin 50% w/v for two hours. At a horizontal laminar air flow cabinet, the fruits were excised from spathes and decontaminated with ethanol 70% for two minutes and calcium hypochlorite 2.5% for ten minutes. After seeds removal, fruits were inoculated in test tubes containing 10 mL of MS basal medium (MURASHIGE & SKOOG, 1962) without growth regulator or supplemented with 2.22µM BA; 8.88 µM BA; 8.88 µM BA + 1.07 µM NAA; 9.05 µM 2.4-D; 18.09 µM 2.4-D.

The explants were kept in a culture room at 24±1ºC and photoperiod of 16 hours. The experimental delineation was entirely randomized with three repetitions of four test tubes. One fruit was put within each tube. The induction of calli was evaluated after 1 month of the inoculation when the explants were transferred to tubes containing MS basal medium with 0,89 µ M BA**.** The morphogenetic responses were evaluated at 1, 2 and 3 months after the fruit inoculation. The results were submitted to analyses of variance and the averages were compared to Tukey's test at 5% of significance.

RESULTS AND DISCUSSION

At the first evaluation, the highest percentages of callus induction in fruit of *A. andreanum* were obtained in the basal media supplemented with 2.22µ M BA and 18.09µ M 2.4-D (Table 1). The increase of BA (MS + 8.88µ M BA) or the reduction of 2.4-D (MS + 9.05 µM 2.4-D) brought down the callus obtainment. Calli were not obtained without the addition of a growth regulator both with or without the combination of BA and NAA. At the second evaluation no callus induction was observed.

The results mentioned for *Anthurium* genus vary in relation to the species, type of explant, and the kind and concentration of the growth regulator. When Kunisaki (1980) tested BA concentrations (0.00, 0.89, 1.78, 2.66, 3.55 and 4.44µ M) in the *A. andreanum* plantlets subculture, the author found that the three higher concentrations were more effective to callus induction, while BA at 0.89µ M promoted the higher clonal multiplication.

TABLE 1 – Percentage of callus induction in *A. andreanum* fruits cultured for 1 month in basal medium MS with and without different types and concentrations of growth regulators. Fortaleza, Embrapa/CNPAT, 2004

Growth Regulators	Callus induction (%)*
—	00.0 c
2.22 µM BA	54.5 a
8.88 µM BA	36.4 b
8.88 µM BA + 1.07 µM NAA	00.0 c
9.05 µM 2.4-D	32.0 b
18.09 µM 2.4-D	63.4 a

*The letters indicate significance by Tukey's test at the level of 5%

Somaya et al. (1998) obtained callus induction in same species through seeds inoculation in MS medium with 9.05µM 2.4-D, and leaves, petioles and roots inoculation in MS medium with 0.45 µM 2.4-D + 4.44µM BA. Hamidah et al. (1997) induced callus from foliar explants of *A. scherzerianum* Schott. in MS medium with 18.09 µM 2.4-D. Atta-Alla et al. (1998) got multiple shoots in seeds of *A. parvispathum* Heml. inoculated in MS medium with 8.88µM BA + 1.07µM NAA.

During the first evaluation, the calli presented a light colour varying from beige to white and they appeared to be organogenic. In the second evaluation almost all calli browned, becoming necrotic. Just one callus kept growing, and it became green and friable with clearly organogenic structures that developed into shoots. This callus was obtained in the medium MS with 18.09µM 2.4-D. At the third evaluation the callus regenerated phenotypically normal entire plants. It is important to point out that fruits are formed from maternal tissues, as well as leaves, different from the seeds that are results of cross fertilisation, allowing uniformity of selected plants.

Although only one callus became organogenic, the results show that it is possible to induce callus in fruit of *A. andreanum* in a short time and at high percentages in media containing relatively low-cost growth regulators. The need to test another media for callus growth, plant regeneration and multiplication is evident.

CONCLUSIONS

It is possible the induction of callus and regeneration of plants from *Anthurium andreanum* fruits.

Callus induction can be achieved by the utilisation of BA or 2.4-D.

ACKNOWLEDGEMENT

To CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico, for financial support.

REFERENCES

ATTA-ALLA, H.; McALISTER, B. G.; STADEN, J. *In vitro* culture and establishment of *Anthurium parvispathum*. **South African Journal of Botany**, Pretoria, v. 64, n. 5, p. 296-298, 1998.

GEIER, T. Anthurium. In: AMMIRATO, P. V.; EVANS, D. R.; SHARP, W. R.; BAJAJ, Y. P. S. (Eds.). **Handbook of plant cell culture**. New York: McGraw-Hill, 1990. v. 5, p. 227-252.

GEORGE, E. F. Micropropagation in practice. In: _____. **Plant propagation by tissue culture**. 2. ed. Somerset: Exegetics, 1996. v. 2, p. 834-1222.

HAMIDAH, M.; KARIM, A. G. A.; DEBERGH, P. Somatic embryogenesis and plant regeneration in *Anthurium scherzerianum*. **Plant Cell, Tissue and Organ Culture**, Dordrecht, v. 48, p. 189-193, 1997.

KUNISAKI, J. T. *In vitro* propagation of *Anthurium andreanum* Lind. **Hortscience**, Alexandria, v. 15, n. 4, p. 508-509, 1980.

LAMAS, A. M. **Floricultura tropical**: técnicas de cultivo e pós-colheita de flores e folhagens. Fortaleza: Instituto Frutal, 2002. 135 p.

MURASHIGE, T.; SKOOG, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. **Physiologia Plantarum**, Copenhagen, v. 15, p. 473-497, 1962.

PIERIK, R. L. M.; STEEGMANS, H.; MEYS, J. A. J. van der. Plantlet formation in callus tissues of *Anthurium andraeanum* Lind. **Scientia Horticulturae**, Amsterdam, v. 2, p. 193-198, 1974.

SOMAYA, K. U.; NARAYANASWAMY, P.; JAYAPRASAD, K. V. Micropropagation studies in *Anthurium andreanum* Lind. **Karnataka Journal of Agricultural Sciences**, Cambridge, v. 11, n. 2, p. 466-470, 1998.

TOMBOLATO, A. F. C.; QUIRINO, E. A.; COSTA, A. M. M. Antúrio (*Anthurium andraeanum* Lindl.). In: TOMBOLATO, A. F. C.; COSTA, A. M. M. **Micropropagação de plantas ornamentais**. Campinas: IAC, 1998. p. 18-21. (Boletim técnico, 174).