Crop Breeding and Applied Biotechnology 9: 274-277, 2009
Brazilian Society of Plant Breeding. Printed in Brazil

NOTE

Embryogenic *calli* induced in interspecific (*Elaeis guineensis* x *E. oleifera*) hybrid zygotic embryos

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Received 12 May 2008

Accepted 30 September 2009

ABSTRACT - *The hybridization between oil palm* (Elaeis guineensis) *and caiaué* (E. oleifera) *plants is directed to obtain progenies presenting high yields like oil palm but with reduced shoot height and resistance to lethal yellowing like caiaué. Cloning F_1, BC_1 and BC_2 progenies can make the replication of selection trials easier. The objective of this work was to induce somatic embryogenesis in interspecific zygotic embryos collected 100 days after pollination. Three progenies were cultivated in an induction medium developed for Tenera* (E. guineensis *tp.* dura *x* pisifera) *embryos. The number of embryos bearing* calli *and germinating was recorded and submitted to the Z test.* Calli *were weighted and submitted to histological analysis. Progenies differed in the number of embryos presenting plumules and* calli *simultaneously. By the ninth month, the apices of incompletely developed somatic embryos were observed protruding from the surfaces of nodular* calli. *Highly embryogenic and friable secondary* calli *producing globular somatic embryos were not observed.*

Key words: Amazon, Arecaceae, oil plants, oil palm, tissue culture.

INTRODUCTION

Oil palm (*E. guineensis* Jacq.) and accessions of caiaué (*Elaeis oleifera* (Kunth) Cortés), the American oil palm, native to the Brazilian Amazon were crossed to obtain plants as productive as *E. guineensis* but resistant to diseases, especially to bud rot and presenting reduced shoot height, which are characteristics from caiaué. F_1 hybrid and BC_1 and BC_2 backcrossed progenies were evaluated as part of the oil palm breeding program conducted at Embrapa Western Amazon (Barcelos et al. 2000).

Cloning is essential to the improvement of these interspecific hybrids. Cloned plants can be used for experimental and commercial plantations. F_1 progenies from selected palms may be cloned to permit the replication of experiments in different locations, reinforcing the representation of the environment throughout the multiple steps of the selection process. It is especially important because *Elaeis* is essentially alogamic and the F_1 progenies can present high variability. Cloning BC_1 and BC_2 progenies can contribute to the accelerate the selection of superior genotypes as well.

The objective of this work was to evaluate the potential of interspecific hybrid zygotic embryos to produce somatic embryos *in vitro*.

MATERIAL AND METHODS

Zygotic embryos came from three different interspecific crosses [SJ 162 (B34-55-18 x RU 49P), SJ

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163 (B34-54-18 x RU 49P) and SJ 165 (B34-55-12 x RU 39P)], between caiaué (*E. oleifera*) feminine progenitors selected amongst the accessions of the germplasm bank (Cunha et al. 2007) and oil palm (*E. guineensis* type *pisifera*) masculine progenitors selected amongst palms used in the production of seeds distributed for commercial plantations. Controlled pollinations were performed at Rio Urubu Experimental Station, Embrapa Western Amazon, Rio Preto da Eva city, the State of Amazonas. Fruits were collected before embryo maturation, 100 days after pollination and the fruit tissues were removed to expose the seeds. At the Plant Biotechnology Laboratory seeds were treated with 50% commercial bleach for 10 min and washed three times in distilled autoclaved water. Embryos were removed from the endosperm, washed in 5% commercial bleach for 3 min and three times in distilled autoclaved water. 100 embryos per progeny were cultivated in Petri dishes (10 per plate) for two months and in 200 mL flasks (5 *calli* per flask) after that. The procedure described by Teixeira et al. (1993) to induce somatic embryogenesis in immature *tenera* (*E. guineensis*, type *dura* x *psifera* commercial hybrids) zygotic embryos was then applied, except that Murashige and Skoog (1962) salts and vitamins were used. The number of embryos developing *calli* and plumules was registered and the results submitted to the Z test (SigmaStat v. 02). Fifteen *calli* from each progeny were weighed from the sixth to the twelfth month. In the sixth month, *calli* were fixed in ethanol 50%: acetic acid 5%: formaldehyde 10%, dehydrated in an ethanol series, treated in tertiary butanol, butanol: chloroform (3:1) and immersed in paraffin to be sectioned. Sections were rehydrated in ethanol, stained with safranin and distained in isopropanol and restained with fast green.

RESULTS AND DISCUSSION

Calli were, independent of the progeny analyzed, induced in the pole opposed to that of shoot emergence. The development of *calli* and plumules were simultaneously observed and there were differences among progenies to this characteristic ($P \leq 0.05$). It was observed more frequently in progeny SJ165 (Table 1) and was reported for Tenera embryos with 77, 91 and 193 days after pollination as well (Teixeira et al. 1993). Roots were observed emerging from the inferior surfaces of some *calli* along the course of the experiments.

Calli from the three progenies grew rapidly in the first months and slowly from the sixth month on. SJ162 and 163 *calli* doubled their average weight, from 600 to 1300 mg from the sixth to the twelfth month. Embryos from progeny SJ165, in addition to present a higher propensity to germinate, produced the smallest and slowest growing *calli*. Taken together these results indicated the occurrence of some influence of the genotypes, which has often been noted in tissue culture experiments (George et al. 1988).

Histological analysis revealed that *calli* cultivated for six months were composed by nodular structures, immersed in a less organized matrix of cells. The nodules, almost round in transversal sections, were considered to be somatic pro-embryos (Figure 1A and B) in their pathway for individualization. Mitotic activity was, apparently, concentrated in the outer layers of each nodule and fused nodules (Figure 1B) were frequently observed. Vascular tissue was visualized in the center of several pro-embryos and structures of proliferation that gave rise to clumps of nodules developed around the vascular axis of zygotic embryos.

One of the developmental patterns described by Schwendiman et al. (1988) for *E. guineensis* embryogenic *calli* led to the production of structures very similar to those described above, that were explained as an "attempt to form a clump of embryos, which nevertheless continued fused". The authors reported that these clumped embryos were able to develop shoots when cultivated under appropriate conditions. In the present work, just the conical apices of the incompletely developed somatic pro-embryos could be seen above the *calli* surfaces, opaque and covered by a brilliant epidermis, from the eighth and ninth months on (Figure 1 C, arrows).

Table 1. Number of immature zygotic embryos from three *E. guineensis* x *E. oleifera* F_1 progenies that developed *calli* and plumules when cultivated for one month in *medium* for the induction of somatic embryogenesis

Progeny	Embryos with *calli*	Embryos with *calli* and plumules	Total
SJ162	99	8	99
SJ163	97	7	99
SJ165	72	23	73

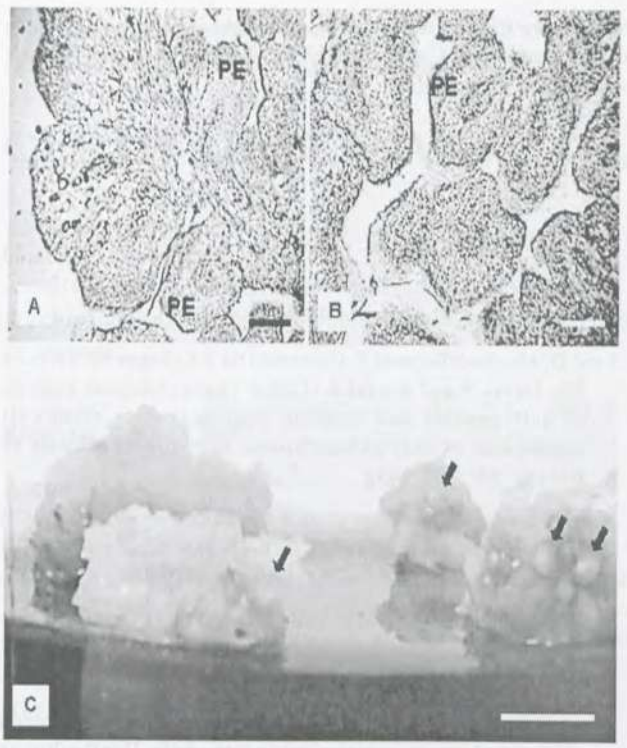

Figure 1. Embryogenic *calli* developed from immature hybrid zygotic embryos obtained from *E. guineensis* x *E. oleifera* interspecific crosses. **A** and **B** histological sections of *calli* cultivated for six months. **A** – longitudinal section showing the proliferation of pro-embryos and a less differentiated mass of tissue, leftmost and **B** – pro-embryos seen in transversal section. **C** – embryogenic *calli* in the ninth month of cultivation. Arrows indicate the apices of incompletely developed pro-embryos. **PE** – somatic pro-embryos. Bars in A and B represent 1 mm. Bar in C represents 1 cm

Plumule-like structures, chlorotic or light green colored, were observed emerging from some of these "cones". This interruption of the individualization process was probably caused by the cultivation in the dark, in absence of shoot inducers, what was expected to promote the development of highly embryogenic sectors over the primary nodular *calli*. The embryogenic sectors would originate pro-embryos in the globular stage, which should individualize *per se* and regenerate complete plantlets, as had been our first expectation because this last pattern of development was observed by Teixeira et al. (1993) for *calli* from Tenera immature embryos. It was reported by Sané et al. (2006) for secondary *calli* of immature date palm leaves too.

In conclusion, the fused pro-embryos we observed arose by a pattern of development completely different from that followed by the highly embryogenic and friable secondary *calli* described by Teixeira et al. (1993). In any case, the results described here can guide future experimentation to bring the interrupted individualization pathway to a better term that would be, for instance, the production of polyembriogenic complexes, similar to those coming from immature *E. guineensis* leaves cultivated in media supplemented by auxins and cytokinins, as described by Hanower and Pannetier (1982), Wong et al. (1997) and Konan et al. (2006). The polyembriogenic complexes are stimulated to produce shoots while embryoids are still fused and the shoots must be excised before rooting.

ACKNOWLEDGMENTS

We are thankful to CNPq for financial support through process 401078/04-3. Thanks to Nelson Lourenço de Paula, Raimundo Oliveira do Nascimento and Raimundo César Pereira de Moraes, Hilma Alessandra Rodrigues do Couto, Karina Pryscilla de Araújo Bichara and Rosimar Fernandes de Souza for kindly manipulating the embryos and to Sérgio Araújo da Silva, for preparing the sections for histological analysis.

Calli embriogênicos induzidos em embriões zigóticos híbridos intereserpecíficos de *Elaeis guineensis* x *E. oleifera*

RESUMO - *Cruzamentos de dendê* (Elaeis guineensis) *com caiaué* (E. oleifera) *visam obter progênies que sejam produtivas como o dendê mas com menor porte e resistentes ao amarelecimento fatal como o caiaué. Clonagem de progênies F_1, RC_1 e RC_2 pode possibilitar a realização de experimentos de seleção com réplicas. O objetivo deste trabalho foi induzir a embriogênese somática em embriões zigóticos híbridos coletados 100 dias depois da polinização. Três progênies foram cultivadas em meio desenvolvido para embriões Tenera* (E. guineensis *tp.* dura *x* pisifera). *O número de embriões apresentando* calli *e germinando foi registrado e submetido ao teste Z.* Calli *foram pesados e submetidos a análise histológica. As progênies diferiram no*

número de embriões com plúmulas e calli *simultaneamente. No nono mês, os ápices de embriões somáticos parcialmente desenvolvidos foram observados projetando-se da superfície dos* calli *nodulares.* Calli *secundários altamente embriogênicos e friáveis produzindo embriões somáticos no estádio globular não foram observados.*

Palavras-chave: Amazonas, Arecaceae, oleaginosas, cultura de tecidos.

REFERENCES

Barcelos E, Nunes, CDM and Cunha RNV (2000) Melhoramento genético e produção de sementes comerciais de dendezeiro. In: Viégas IJ and Müller AA (eds.) **A cultura do dendezeiro na Amazônia brasileira**. Embrapa Amazônia Oriental/ Embrapa Amazônia Ocidental, Belém, p. 145-174.

Cunha RNV, Lopes R, Sousa NR, Teixeira PC and Rocha RC (2007) Produção de cachos de caiaué da Amazônia brasileira. In: **4º Congresso Nacional de Melhoramento de Plantas**. Sociedade Brasileira de Melhoramento de Plantas, Lavras. (CD-ROM).

George EF, Puttock DJM and George HJ (1988) **Plant media culture**. Exegetics, Edington, p. 391-397.

Hanower J and Pannetier C (1982) *In vitro* vegetative propagation of the oil palm *Elaeis guineensis* Jacq. **Plant Tissue Culture SP**: 745-746.

Konan KE, Durand-Gasselin T, Kouadio YJ and Rival A (2006) A modeling approach of the *in vitro* conversion of oil palm (*Elaeis guineensis* Jacq.) somatic embryos. **Plant Cell Tissue Organ Culture 84**: 99-112.

Murashige T and Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. **Physiologia Plantarum 15**: 473-497.

Sané D, Aberlenc-Bertossi F, Gassama-Dia YK, Sagna M, Trouslot MF, Duval Y and Borgel A (2006) Histocytological analysis of callogenesis and somatic embryogenesis from cell suspensions of date palm (*Phoenix dactylifera*). **Annals of Botany 98**: 301-308.

Schwendiman J, Pannetier C and Michaux-Ferriere N (1988) Histology of somatic embryogenesis from leaf explants of the oil palm *Elaeis guineensis*. **Annals of Botany 62**: 43-52.

Teixeira JB, Söndahl MR and Kirby EG (1993) Somatic embryogenesis from immature zygotic embryos of oil palm. **Plant Cell Tissue and Organ Culture 34**: 227-233.

Wong G, Tan CC and Soh AC (1997) Large scale propagation on oil palm clones – experiences todate. **Acta Horticulturae 447**: 649-658.