01-145- SOMATIC EMBRYOGENESIS IN ASSAI PALM (*Euterpe oleracea* Mart.).

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ABSTRACT

Among several native species of the Amazon area stands out the assai palm, due to the different use possibilities and the great potential of commercialization of its products and by-products, in the national and international markets, particularly the "palmito" (heart of palm). However, inadequate techniques of propagation and absence of improved genetic material, have contributed negatively to the rational and economic exploitation of this species. No information about assai palm micropropagation is available until now. The aim of the present work was the production of mature somatic embryos and the recovery of entire plants. The research experiments were conducted, in laboratory, mature fruits were collected from of assai palm plants at Embrapa Amazônia Oriental, Belém-PA, Brazil. The mature zygotic embryos were excised from mature seeds surface-disinfected in ethanol at 70% for two minutes followed by immersion in solution of sodium hypochloride at 2% for 20 minutes. Seeds were rinsed with four changes of sterile water. The zygotic embryos were removed from the seeds and transferred onto induction medium which consisted of MS medium (Murashige and Skoog, 1962) supplemented with agar (6 $g.L^{-1}$), activated charcoal (2,5 $g.L^{-1}$), sucrose (30 $g.L^{-1}$), casein hydrolysate (500 $mg.L^{-1}$) and 2,4-D (25; 50; 75; 100; 125 e 150 $mg.L^{-1}$). It was possible to verify the expression of a direct, repetitive and asynchronized model of somatic embryogenesis in mature zygotic embryos cultivated in primary MS medium supplemented with 2,4-D (100 $mg.L^{-1}$) and transferred to a secondary MS medium in the presence of NAA (0,1 $mg.L^{-1}$) and 2iP (2,5 $mg.L^{-1}$). The conversion of embryos in plantlets was obtained after the transfer of somatic embryos to a third medium with sucrose and mineral salts reduced to the half-strength of the basal medium, in the absence of the growth regulators.

INTRODUCTION

Among several native species of the Amazon area stands out the assai palm, due to the different use possibilities and the great potential of commercialization of its products and by-products, in the national and international markets, particularly the "palmito" (heart of palm). However, inadequate techniques of propagation and absence of improved genetic material, have contributed negatively to the rational and economic exploitation of this species. No information about assai palm micropropagation is available until now. The studies have been achieved for *Cocos nucifera, Phoenix dactilyfera* and *Elaeis guineensis* that present great economic impact in the international markets. In general, two basic procedures have been used for palm tissue culture: somatic embryogenesis and the reversion of young flower meristem to a vegetative state (Tisserat, 1987). Guerra and Handro (1991, 1998) obtained with success the direct somatic embryogenesis starting from zygotic embryos and young inflorescences of *Euterpe edulis* Mart. The aim of the present work was the production of mature somatic embryos and the recovery of entire plants.

MATERIAL AND METHODS

The research experiments were conducted, in laboratory, mature fruits were collected from of assai palm plants at Embrapa Amazônia Oriental, Belém-PA, Brazil. The mature zygotic embryos were excised from mature seeds surface-disinfected in ethanol at 70% for two min followed by immersion in solution of sodium hypochloride at 2% for 20 min and rinsed in sterilized distilled water. The zygotic embryos were removed from the seeds and transferred onto induction medium which consisted of MS medium (Murashige and Skoog, 1962) supplemented with agar (6 $g.L^{-1}$), activated charcoal (2.5 $g.L^{-1}$), sucrose (30 $g.L^{-1}$), casein hydrolysate (500 $mg.L^{-1}$) and 2,4-D (25; 50; 75; 100; 125 e 150 $mg.L^{-1}$). The pH was adjusted to 5.8 after autoclaving. The embryogenic cultures induced in the primary medium, were transferred for secondary MS medium with agar (6 $g.L^{-1}$), sucrose (20 $g.L^{-1}$), in the presence of NAA (0.1 $mg.L^{-1}$) combined with 2iP (2.5 $mg.L^{-1}$), with the objective of to stimulate to the progression of the initial phases for late phases and to obtain mature somatic embryos. For the somatic

embryo conversion and plantlet development, the cultures were transferred for ½ MS medium, with agar (6 $g.L^{-1}$) and sucrose (15 $g.L^{-1}$), in the absence of plant growth regulators. In the initial period of 7 days, the cultures were maintained in the dark to prevent browning, with temperature of 26 ± 2ºC, relative humidity of the air average around 70%. After this period cultures were transferred to a light/dark cycle, 16/8 hr, photonic flux ca. 52 $\mu mol.m^{-2}.s^{-1}$.

RESULTS AND DISCUSSION

When zygotic embryos are cultured in increasing concentrations of 2,4-D (75-125 $mg.L^{-1}$), distinct morphogenetic responses occurred (Table I). It was possible to verify the progressive inhibition of germination, and the development of granular structures on the cotyledonar node. These granular structures developed in translucid structures that characterize the initial stages of somatic embryogenesis. The matrix tissue produces new embryos, which remain at the globular stage, and a continuous and non-synchronous embryogenic process continues. The transference of post-globular embryos to the same basal medium in the absence of activated charcoal and supplemented with 2-iP and NAA resulted in the progression to a bipolar stage. The conversion of embryos in plantlets was obtained after the transfer of somatic embryos to a third medium with sucrose and mineral salts reduced to the half-strength of the basal medium, in the absence of the growth regulators. These results showed that a high frequency, direct, non-synchronous and continuous model of somatic embyogenesis could be obtained in cultures of zygotic embryos of *Euterpe oleracea* Mart., leading to whole normal plantlets. Guerra and Handro (1988; 1998) described similar model of somatic embryogenesis in immature embryos of *Euterpe edulis* Mart.

CONCLUSIONS

It was possible to obtain the expression of a direct, repetitive and non-synchronized model of somatic embryogenesis in mature zygotic embryos of *Euterpe oleracea* Mart.

Table 1. Morphogenetic responses of mature zygotic embryos of *Euterpe oleracea* Mart., cultivated in MS medium supplemented with different concentrations of 2,4-D, at the 80 days of culture.

2,4-D ($mg.L^{-1}$)	Nº explants	Nº explants with morphogenetic responses	% germination[1]	% explants with granular structures [1]	% explants with globular embryos [1]
25	45	36	100,0	0	0
50	45	29	100,0	0	0
75	45	39	0	80,43	19,57
100	45	27	0	61,42	38,58
125	45	17	0	93,41	6,59
150	45	0	0	0	0

[1] % on the total explants with morphogenetic responses

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