Somatic embryogenesis and plant regeneration in embryo cultures of *Euterpe edulis* mart. (palmae)

Miguel Pedro Guerra and Walter Handro

Plant Tissue Culture Laboratory, Department of Botany, Institute of Biosciences, University of São Paulo, 05499 São Paulo, Brazil

Received September 7, 1988/Revised version received October 12, 1988 - Communicated by I.K. Vasil

**ABSTRACT**

The induction of somatic embryogenesis in embryo cultures of *Euterpe edulis* is described. The basal medium was composed of LS salts and Morel & Wetmore vitamins. Activated charcoal was added to prevent explant oxidation. 2,4-D higher than 50 mg/l was necessary for inducing embryogenesis which occurs 45-180 days after the start of cultures. Embryos arise directly from surface proliferating tissues on the matrix structure, without callus formation. The transfer of tissues with embryo clusters to medium with NAA plus 2iP, or without growth regulators, induces embryo development into plantlets.

Key words: LS: Linsmaier & Skoog; 2,4-D: 2,4-dichlorophenoxyacetic acid; 2iP: 2-isopentenyladenine; NAA: naphthaleneacetic acid.

**INTRODUCTION**

The use of in vitro techniques for morphogenetic studies and micropropagation in neotropical palms has increased significantly, especially in species with high economic interest such as *Phoenix dactylifera*, *Elaeis guineensis* and *Cocos nucifera* (see Reynolds 1982, Tisserat & Pannetier & Buffard-Morel 1986). The palm *Euterpe edulis* is indigenous to a narrow area of moist forests in Southern and Southeastern Brazil; its main product, the heart of palm, has an increasing world consumption. This rising demand is threatening its extinction, because to get the heart, the tree must be killed. Here we describe the establishment of embryo cultures of *E. edulis* and plant regeneration.

**MATERIAL AND METHODS**

Embryos were isolated from mature and immature fruits from plants occurring naturally in forests in the State of São Paulo. Before embryo isolation, fruits were sterilized for 12 h in a solution having 4% sodium hypochlorite, 0.15% streptomycin and 0.01% merthiolate, on a reciprocal shaker. Fruits were then immersed in 70% ethanol for 5 min, and finally rinsed in sterile water. The basal medium was composed of LS salts (Linsmaier & Skoog 1965), vitamins (Morel & Wetmore 1951), 0.15% activated charcoal, and 3% sucrose. Growth regulators (2,4-D, NAA, 2iP) were used at several concentrations depending on the stage of the cultures. The pH was adjusted to 5.5 and the medium gelled with 0.45% Merck agar before autoclaving. The cultures were incubated at 26°C in 16 h/day of light (Gro-Lux lamps, 5 W.m⁻²).

**RESULTS AND DISCUSSION**

The success in the maintenance of viable embryo cultures depended entirely on rapid excision and inoculation, and the presence of activated charcoal in the media. Consistent and repetitive morphogenetic responses were observed when 2,4-D was added to the basal medium at different concentrations; until 50 mg/l embryos developed similarly as in normal germination, forming the cotyledonal petiole and the haustorium. In cultures with 100 mg/l 2,4-D, a granular tissue develops showing red (from the cotyledonal petiole) and white sectors (from the haustorium). After periods ranging from 45 to 180 days in culture, globular-translucent structures were seen on the cotyledonal petiole tissue (Fig. 1). These structures, typically embryogenic, increased rapidly in size and number and up to 50 of them could be seen in several stages of development (Fig. 2). Later, pearly-stage embryoids attached to the matrix through narrow suspensors were...
formed (Fig. 3). The isolation of this mass of embryos and transfer to medium devoid of 2,4-D (solid or liquid, with or without charcoal) caused the development of the embryos into plantlets (Fig. 4), but better results were obtained when the cultures having embryogenic mass were transferred to medium containing 2iP (5 mg/l) plus NAA (0.5 mg/l) followed by transfer to medium devoid of growth regulators. The somatic embryo masses are always formed on compact regions of the tissues from the cotyledonary petiole, never from haustorium tissues. Moreover, embryos excised from immature fruits were more suitable for somatic embryogenesis.

In monocotyledons, organogenesis and embryogenesis are two possible morphogenetic routes for obtaining plants in vitro. Embryogenesis seems to be the most important pathway in Palmae (Blake 1983) and Gramineae (Vasil & Vasil 1982). Our results show clearly the occurrence of somatic embryogenesis in *Euterpe edulis* embryo cultures with liberation of bipolar embryos from the matrix structure. Further development of the somatic embryos into plantlets is similar to the development of zygotic embryos and subsequent germination as described by Bellin-Depoux and Queiros (1971).

In most plant species and specially in monocotyledons, 2,4-D is responsible for conferring embryogenic competence to tissues cultured in vitro (Ammirato 1983). As reported by many previous workers, the stage of development of the explants is particularly important in the embryogenic process. Somatic embryogenesis may be a consequence of a subtle interaction between the developmental stage and 2,4-D concentration. If adequate this interaction will result in the suppression of zygotic embryo growth and induction of somatic embryogenesis with the new embryos arising directly on the cotyledonary petiole surface, showing a clear difference with other palms, where embryos originate from callus (Tisserat 1984). In *E. edulis* true calluses were not formed and probably a model of direct embryogenesis occurs suggesting an origin from single superficial cells where embryos are linked to the matrix through suspensors (see Williams & Maheswaran 1986). This seems to be in accordance with observations of Sharp et al. (1980) and Evans et al. (1981) that direct embryogenesis occurs from cells determined previously before in vitro culture, the only necessity being growth regulators and other special conditions for cell division.