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Callus Formation from Inflorescences in Rhapis excelsa

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ABSTRACT

The purpose of this study was to develop a method for vegetative propagation of *Rhapis* palm through tissue culture. Young inflorescences were used as an explant source. For callus induction different concentrations of auxins were evaluated. Callus was induced on most of the inflorescences tissues with the highest concentrations of growth regulators.

Rhapis are decorative, small, clump-forming fan palms, useful for planting in many situations, as they have a graceful shape and may be used both outdoors and indoors, growing in low light conditions. *Rhapis excelsa* is one of the best garden palms but because of its very slow growth plants tend to be expensive and therefore the species is not widely planted (Jones 1984). Some larger specimens or some rare species, such as the variegated *Rhapis* are worth several thousands dollars (Reynolds 1982).

Most propagation is by division of the clumps, but each mature palm produces an average of two offshoots per year (McKamey 1983). Seeds are not usually available (Rodríguez 1994). "However, they are more readily available than in 1983. The problem is still finding reliable seed sources which will supply true *R. excelsa,* not *R. subtilis* or *Guihaia.* A seedling takes three years to develop mature leaves, and only then can it be correctly identified as *R. excelsa.* Many growers have made quite an investment in seed, only to discover years later that they did not have *R. excelsa*" (L. McKamey, pers. comm.). When seeds are germinated in the usual manner, this process takes two to three months or more, depending upon temperature (Blombery and Rodd 1982). Several researchers interested especially in palms have developed protocols for clonal propagation of oil palm (*Elaeis guineesis* Jacq.), date palm (*Phoenix dactylifera* L.) and coconut (*Cocos nucifera* L.) using tissue culture technology. The aim of this study was to evaluate the effect of auxins on callus formation and plant regeneration to find a more efficient method for clonal propagation.

The inflorescences, which arise from the upper leaf sheaths, are male and female on separate plants and usually pinkish at the beginning, turn light green and finally white at anthesis (Blombery and Rodd 1982; L. McKamey, pers. comm.). Pollination requires both male and female plants (McKamey 1989). For collecting immature *Rhapis* inflorescences we used a technique developed by Rillo (1989) to collect nondestructively immature coconut inflorescences for tissue culture purposes. The inflorescences were surface sterilized by immersing them for 20 min in 2% (w/v) calcium hypochlorite solution and adding three rinses in autoclave distilled water after the sterilization.

The outermost sheath of leaves was carefully removed under sterile conditions and discarded. Inflorescences were sliced into 1–2 mm sections and inoculated onto MS medium (Murashige and Skoog 1962), supplemented with auxin and activated charcoal. The pH of the medium was kept at 5.6–5.8 by using sodium hydroxide prior to adding agar. Medium was sterilized in an autoclave at 121°C and 1.2 kg/cm^2 for 15 minutes. Explants were cultured in a growth chamber at 25° ± 2°C under dark.

As reported for many palms, *Rhapis* callus production was very slow. The first sign of callusing (floral bud swelling) becomes evident after three months (Verdeil et al. 1994). When the callus was transferred to medium with a reduced auxin concentration the development increased and produced more defined white structures, ensuring calloid growth. The results showed that auxin, at selected levels, promoted callus formation on immature inflorescences of *Rhapis excelsa.* After three months of incubation in the dark, calli were white to pale yellow in color and had a hard con-

1. Callus formed from immature inflorescences of *Rhapis excelsa.*

sistency (Fig. 1.) Several tissues from mature donor plants have been used as starting explants in reliable protocols for palm plant regeneration. Immature inflorescences represent an important explant source for somatic embryogenesis of oil and coconut palm trees (Texeira et al. 1994, Verdeil et al. 1994).

The work with oil palms shows that other palms may be similarly propagated by tissue culture. The results presented here proved that callus can be induced and multiplied from inflorescence explants of *Rhapis* palm. Success in obtaining callus was probably the result of using very young inflorescences and the presence of a suitable concentration of auxin and activated charcoal. Similar results were achieved for male and female inflorescences, although male ones formed calli in all auxin concentrations. However, female inflorescences responded only to the highest levels of these plant growth regulators.

Histological studies performed by Verdeil et al. (1994) showed that globular white callus was formed from male floral meristems. They observed a very highly significant influence of 2,4-D concentration on the percentage of explants bearing callus. The auxin concentration was reduced during several subcultures followed by addition of BAP. Calli produced in cultures derived from immature inflorescences of *Rhapis* palm was similar to those observed in callus lines derived from immature inflorescences of coconut (Verdeil et al. 1994) and oil palm (Teixeira et al. 1994).

Micropropagation should find a ready application in the coconut, date, and oil palm industries and in the production of desired ornamental palms. Only the techniques of micropropagation will allow a rapid increment in sufficient number of *Rhapis* palms. Vegetative multiplication of the individual remains a promising possibility for the production of homogeneous planting material and for substantial improvement in plant homogeneity.

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