Abstract. Excised zygotic embryos of Christmas palm, Agriculture Sciences, University of Florida, 18905 SW 280 Street, Homestead, FL 33031, tissue culture, embryoid, in vitro.

Additional index words. tissue culture, embryoid, in vitro

Literature Cited

Somatic Embryogenesis and Plantlet Formation from Christmas Palm Callus

C. Srinivasan1, R.E. Litz, J. Barker, and Knut Norstog2 Tropical Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, 18905 SW 280 Street, Homestead, FL 33031

Additional index words. tissue culture, embryoid, in vitro

Abstract. Excised zygotic embryos of Christmas palm [Veitchia merrilli (Bacc.) H.E. Moore] developed large haustoria and germinated normally in vitro on Murashige and Skoog (MS) medium plus 0.25% activated charcoal, 5-50 μM 2,4-D and 5 μM BA. Embryogenic callus was induced from mature zygotic embryos when they were cultured on charcoal-free MS medium supplemented with 170 mg/liter KH₂PO₄, 200-400 mg/liter glutamine, and 5-25 μM 2,4-D. Somatic embryos developed and matured in a hormone-free, glutamine-containing medium. Plantlets developed from somatic embryos on MS basal medium or MS plus 5 μM NAA, 5 μM 2 iP, and 2.5 μM GA₃. Embryogenic calli have been maintained on MS medium for more than 6 months. Chemical names used: (2,4-dichlorophenoxy)acetic acid (2,4-D); N-(phenylmethyl)-1H-purin-6-amine (BA); 1-naphthaleneacetic acid (NAA); N-(3-methyl-2-butenyl)-1H-purin-6-amine (2IP).

Christmas (Manila) palm is an important ornamental palm in south Florida and in other tropical regions. This palm is heterozygous, arborescent and unbranched, and it is seed propagated. About 30 palm species including Christmas palm, coconut, and date palms are affected by the devastating disease “lethal yellowing” (LY) which has almost completely destroyed these palms in south Florida and in Caribbean countries (5). This disease is believed to be caused by a mycoplasma-like organism that has been found in LY-affected palms (5). Continuous feeding of the antibiotic oxytetracycline through the palm trunk is the only available method to reduce LY-symptoms, but even this treatment is expensive and frequently ineffective. Therefore, we initiated tissue cultures of Christmas palm to explore the possibility of selecting somaclonal variants tolerant or resistant to LY disease. In addition, regeneration of plants by tissue culture is the only feasible method of cloning these palms.

Mature fruit (2-3 cm long) of the Christmas palm were harvested during January to March of 1983 from open-pollinated palm collections of Fairchild Tropical Garden in Miami. The fruit are hard to cut with a scalpel or a knife. After surface-sterilizing with 1.25% sodium hypochloride for about 30 min, the fruit were rinsed once with sterile water and split into 2 longitudinal halves with a presterilized nut cracker. The zygotic embryos then were excised with a scalpel. The zygotic embryos were cultured in glass test tubes on 10 ml MS medium (6) supplemented with 170 mg/liter potassium dihydrogen phosphate, 5-500 μM 2,4-D, 2,4,5-T, (2-naphthalenyl)oxycetic acid (BNOA), (4-chlorophenoxy)acetic acid (4CPA).

Some media contained 200-400 mg/liter glutamine, or 5-15 μM 2IP, 5μM BA, or 0.25% activated charcoal. All media were adjusted to pH 5.7 before autoclaving and were gelled with 0.7% Difco Bacto agar. The cultures were maintained in a growth chamber providing 24 μmol m⁻² s⁻¹ light from Gro-Lux fluorescent tubes with a 16 hr photoperiod at 27°C, or under complete darkness.

Twenty embryos were cultured in each concentration of the auxins used. Seventy to 100% of the zygotic embryos on media containing 5-50 μM auxin, 5 μM BA, or 2IP and 0.25% activated charcoal germinated. Initially, there was enormous growth of the haustorium followed by the appearance of a shoot and root. The haustoria enlarged more than 100 times in 5-9 weeks after culture. The haustorium was white in dark-grown plants and turned green within a week after exposure to light. The enlargement of haustoria was affected by the type and con-
2.4-D 2,4,5-T NOA 4-CPA

1. Effect of auxins on the germination and development of haustoria of *Veitchia* zygotic embryos (×0.2). 2. Formation of nodular embryogenic callus derived from zygotic embryos 8 weeks after culture in MS plus 400 mg/liter glutamine (×20). 3. Embryogenic clump showing various stages of somatic embryo development in MS plus 400 mg/liter glutamine (×10). 4. Germination of *Veitchia* plantlet in MS plus 5 μM NAA, 5 μM 2iP, and 2.5 μM GA 7 weeks after incubation (×1). 5. Multiple adventitious shoot formation from a somatic embryo 11 weeks after culture in the same medium given in Fig. 4 (×1). 6. A *Veitchia merrilli* plantlet 5 weeks after transplanting into non sterile peat:perlite mixture (×0.5).

Concentration of auxin in the media. The most extensive enlargement of the haustoria was observed on medium containing 5 μM 2,4-D, 5 μM BA, and 0.25% activated charcoal (Fig. 1). The size (length and diameter) of the haustoria was progressively reduced in auxin concentrations greater than 50 μM. Mature zygotic embryos cultured on charcoal-free media containing 5–25 μM 2,4-D.
Table 1. Effect of tissue culture media on somatic embryogenesis from Christmas palm callus 10 weeks after incubation. Fourteen to 23 cultures were evaluated.

<table>
<thead>
<tr>
<th>Media</th>
<th>No. of embryogenic cultures (% of total)</th>
<th>Mean no. of embryos/culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS</td>
<td>76</td>
<td>22 ± 6</td>
</tr>
<tr>
<td>B5</td>
<td>68</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>Schenk and Hildebrandt</td>
<td>57</td>
<td>32 ± 7</td>
</tr>
<tr>
<td>N6</td>
<td>49</td>
<td>41 ± 4</td>
</tr>
<tr>
<td>Nitsch &amp; Nitsch</td>
<td>52</td>
<td>48 ± 6</td>
</tr>
</tbody>
</table>

and 200–400 mg/liter glutamine enlarged initially, and then began to produce creamy white or yellowish nodular callus from the germ pore end, both in darkness and in light. Subculture of 50 to 60 mg of these nodular calli onto basal MS medium containing 200–400 mg/liter glutamine produced more friable, dense white callus and translucent somatic embryos (Fig. 2). Embryogenic callus of Christmas palm can be maintained for up to 6 months on an MS medium plus 5 μM 2,4-D mg/liter glutamine, without subculture. Somatic embryos also have been produced from mature zygotic embryos of other *Veitchia* spp., i.e., *Veitchia montgomeryana* and *V. macdanielsii*. The growth of embryogenic callus appeared to be higher on MS and B5 media (4), but the number of fully-developed embryos was increased on the media of Schenk and Hildebrandt (10), Nitsch and Nitsch (7), and N6 (3) (Table 1). Asexual embryos produced on MS and B5 media were about twice as large as those that developed on other media.

Most somatic embryos remained white even under light. Somatic embryogenesis was not synchronous. Several stages of embryo development have been observed in a single clump (Fig. 3). A similar situation also has been reported in cereals and grasses (12). The embryos have been transferred to MS medium supplemented with various growth regulators to induce plantlet formation. On MS basal medium or MS with 5 μM NAA, 5 μM 2iP, and 2.5 μM GA₃, somatic embryos germinated after 7–10 weeks under light (Fig. 4). Some embryos produced multiple secondary shoots which appeared to be adventitious in origin (Fig. 5). *Veitchia* palms do not normally produce axillary shoots. When the plantlets were 5 cm high or larger, they were transplanted into a 1:1 nonsterile peat moss: perlite (v/v) mixture (Fig. 6) and were irrigated with mineral nutrient solution to prevent dessication.

In contrast to date palm tissue cultures, in which embryonic callus has been produced by culturing immature zygotic embryos on medium containing a high 2,4-D concentration (450 μM) and in the presence of activated charcoal (9), *Veitchia* embryogenic calli have been induced from mature zygotic embryos with low concentrations (5 μM) of 2,4-D on charcoal-free medium. However, the patterns of callus induction, somatic embryogenesis, and plantlet formation are similar to other palm species (2, 8, 11). The formation of adventitious shoots from germinating embryos also has been observed from cultured, germinating zygotic embryos of coconut (1). Multiple shoots also are observed commonly during the germination of grass somatic embryos (12).

It is hoped that embryogenic calli, somatic embryos, and plantlets of *Veitchia* can be used to study the host-pathogen relationship between LY-causing mycoplasma and palm cells. Moreover, the regeneration procedure described here may be useful for large scale clonal multiplication of this important ornamental palm.

**Literature Cited**