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Plantlet Regeneration from Shoot Tip Cultures of *Xanthosoma caracu*

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Xanthosoma, Araceae (tannia, cocoyam, yautia) is an underexploited root crop cultivated in tropical America, Africa, Asia, and Polynesia as a subsistence food (Anonymous, 1975; Kay, 1973). Many, perhaps most, cultivated *Xanthosoma* plants are infected with virus(es). This infection persists because the crop is propagated vegetatively. Propagation by seeds (Hartman *et al.*, 1972) produces virus-free plants, but is inappropriate for agricultural purposes because it is sexual and therefore unsuitable for the maintenance of clones. In contrast, aseptic culture techniques can be used to produce virus-free clonal material for both research and agricultural purposes (for a review see Arditti and Strauss, 1979). Such procedures have been developed for *X. sagittifolium* (Hartman, 1974; Lot *et al.*, 1974) and *X. brasiliensis* (Staritsky, 1974), but not *X. caracu* (Volin and Zettler, 1975).

Shoot tips including 2–3 leaf primordia were excised from corms provided by Dr F. W. Zettler, University of Florida, Gainesville. Corms, surface sterilized with 50 per cent (v/v) aqueous household bleach (5·25 per cent sodium hypochlorite), and the larger outer leaves were removed with sterile forceps. The last few leaves were cut away using a sterile micro-scalpel (devised by Dr E. A. Ball; for details on its construction see Arditti and Strauss, 1979) thus exposing the meristem. Shallow incisions were made on all sides of the meristem, a cube of tissue (0·3–0·5 mm^3) was removed and cultured in 25×250 mm tubes containing 20 ml medium solidified with 1·0–1·2 per cent agar.

Explants were cultured on Linsmaier–Skoog (LS) medium (Linsmaier and Skoog, 1965) supplemented with 1 mg l^{-1} of the synthetic cytokinin SD 8339 [adenine, N-benzyl-9(tetrahydro-2Hpyran-2-yl) provided by Dr Juan G. Morales, Shell Development Company, Modesto, CA 95352, U.S.A.] and 0·1 mg l^{-1} naphthaleneacetic acid (NAA). Callus cultures were also maintained on this medium by the use of 1 mg l^{-1} kinetin instead of SD 8339. A medium containing 1 mg l^{-1} kinetin, 2 mg l^{-1} NAA, 1 mg l^{-1} spermine tetrahydrochloride, (Sigma), and 5 per cent (v/v) coconut water from green nuts was used to enhance shoot production. All media were sterilized by autoclaving.

To initiate callus, explants were maintained in darkness for 2–3 months. The cultures were transferred to light when callus masses exceeded *c.* 5 mm in diameter. All cultures were maintained at 22 ± 2 °C. Photoperiods were 18 h provided by a mixture of 25 W incandescent bulbs and 40 W Sylvania Gro Lux fluorescent tubes. The light intensity was approximately 1600 lx.

Plantlets were transferred to the greenhouse when they exceeded 10 cm in height. The

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potting mixture consisted of redwood compost, peat moss and sand (1:2:3 by volume) and was steam sterilized. To ensure their survival and prevent dessication, the plantlets were covered with plastic sheet or placed in shade for the 7 days. Humidity in the greenhouse was 60–65 per cent and temperatures ranged from 29 °C during the day to 22 °C at night.

In several instances only a single plantlet was produced by each explant. However, most explants formed callus within a month. On transfer to fresh medium (usually after 4–6 weeks) the callus continued to grow reaching a diameter of *c.* 5 mm in 2–3 months. The callus masses were white and, except for an occasional shoot, undifferentiated (Plate 1 A). Following sub-culture or transfer to the light the callus turned green within 7 days and shoot formation increased. The callus was maintained by sub-culturing 3–5 mm^3 sections to fresh medium every 4–6 weeks.

Plantlet formation occurred when the callus was not sub-cultured for more than 4–6 weeks. Generally one to three plantlets were formed in each tube (Plate 1 C) after 8 weeks. The number of shoots was greater (five to seven per tube) in the presence of 1 mg l^{-1} kinetin, 2·5 p.p.m. NAA, and spermine tetrahydrochloride (Plate 1 B, D). No tests were carried with a similar medium lacking spermine. Once formed, plantlets grew equally well on all media listed in the materials and methods section and were ready for potting by 12–14 weeks. On being planted in potting mix and transferred to the greenhouse the plantlets grew normally and appeared healthy.

The callus could be maintained by regular sub-culture. However work with *Colocasia* indicates that plants from callus which has been sub-cultured repeatedly may be morphologically, biochemically and cytologically different from the original clone. We do not know whether the same is true for *Xanthosoma.*

Of the eight edible *Xanthosoma* species, seven, including *X. caracu* and *X. brasiliensis*, are from tropical America; one, *X. sagittifolium*, is from Africa (Purseglove, 1972). However, it has been suggested that 'until such time as further critical taxonomic studies have been made, it seems better to lump most of the cultivars in one polymorphic species, *X. sagittifolium*, a practice commonly resorted to by most agriculturists' (Purseglove, 1972). This suggestion is not universally accepted and *X. caracu* is grown as a separate species in Puerto Rico (Kay, 1973). In Florida *X. caracu*, *X. atrovirens* and *X. violaceum* are recognized as separate species; *X. sagittifolium* does not occur there (Volin and Zettler, 1975). Therefore it appears reasonable to treat *X. caracu* as a separate species.

Cultivated aroids (edible and ornamental) differ in the ease with which they can be cultured (Arditti and Strauss, 1979). This is especially true for taro, *Colocasia esculenta* var *esculenta* and *C. esculenta* var *antiquorum* (Jackson, Ball and Arditti, 1975, 1977*b*) and may also be the case with *Xanthosoma* (Arditti and Strauss, 1979). Therefore, in the absence of published methods for the culture of *X. caracu*, we employed and modified media and approaches which were successful with taro (Jackson, Ball and Arditti, 1975, 1977*a*, *b*; Strauss, Michaud and Arditti, 1979).

SD 8339 is an experimental cytokinin which is very useful (Jackson, *et al.*, 1977*b*; Pierik, Steegmans and van der Meys, 1974), but may no longer be available. Therefore, we replaced it with kinetin which has been used in cultures of *X. sagittifolium* (Hartman, 1974) and *X. brasiliensis* (Staritsky, 1974). Benzyladenine (BA) has also been used successfully in *X. brasiliensis* cultures (Staritsky, 1974).

Spermine has been reported to enhance growth in *Helianthus tuberosus* (Bertossi *et al.*, 1965) while spermine tetrahydrochloride increased the number of buds formed on *Sequoia sempervirens* stem explants *in vitro* (E. A. Ball and D. M. Morris of this Department, personal communication). It appears to have similar effects on *Xanthosoma.* Spermine-containing media were autoclaved because this mode of sterilization does not seem to have deleterious effects on its desired activity which is to increase the number of plantlets.

Dasheen Mosaic Virus (DMV) is widespread in *Xanthosoma* planting (Volin and Zettler, 1975) and perpetuated by current agricultural practices. To reduce its incidence or eliminate it, virus-free planting stock must be used. Shoot tip cultures are used widely to free plants from virus infections (Arditti, 1977; Gamborg and Wetter, 1975; Reinert and Bajaj, 1977; Street, 1977) and have been employed with aroids (Abo El-Nil and Zettler, 1976; Hartman, 1974; Jackson *et al.*, 1977*b*), including *Xanthosoma* (Staritsky, 1974; Volin and Zettler, 1975). Their use to free *X. caracu* from DMV has been suggested (Volin and Zettler, 1975), but, as far as we can determine, not yet accomplished. The method described here may be used to achieve this goal.

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EXPLANATION OF PLATE

Regeneration of *Xanthosoma caracu* plantlets from callus cultures of shoot tip origin.

A. Unorganized callus growing on shoot induction medium containing 1 mg l^{-1} NAA. ×49. Callus is maintained by sub-culture or transfer every 4 to 6 weeks on the same medium.

B. Callus, initiated on the above medium, 7–14 days after sub-culture to a medium containing 1 mg l^{-1} spermine tetrahydrochloride and 5 per cent liquid endosperm from unripe coconuts. Note the numerous small plantlets which have formed. ×32.

C. Representative culture showing plantlet formed after 12–14 weeks on a medium containing 1 mg l^{-1} SD 8339 and 0·1 mg l^{-1} NAA. ×1·8. Similar results can be obtained on a medium containing 1 mg l^{-1} kinetin instead of SD 8339.

D. Representative culture showing multiple plantlets 6–7 weeks after callus was sub-cultured or transferred to a medium containing 1 mg l^{-1} spermine tetrahydrochloride and 5 per cent liquid endosperm from green coconuts. ×1·5. Note the larger number of plantlets formed when compared to C.

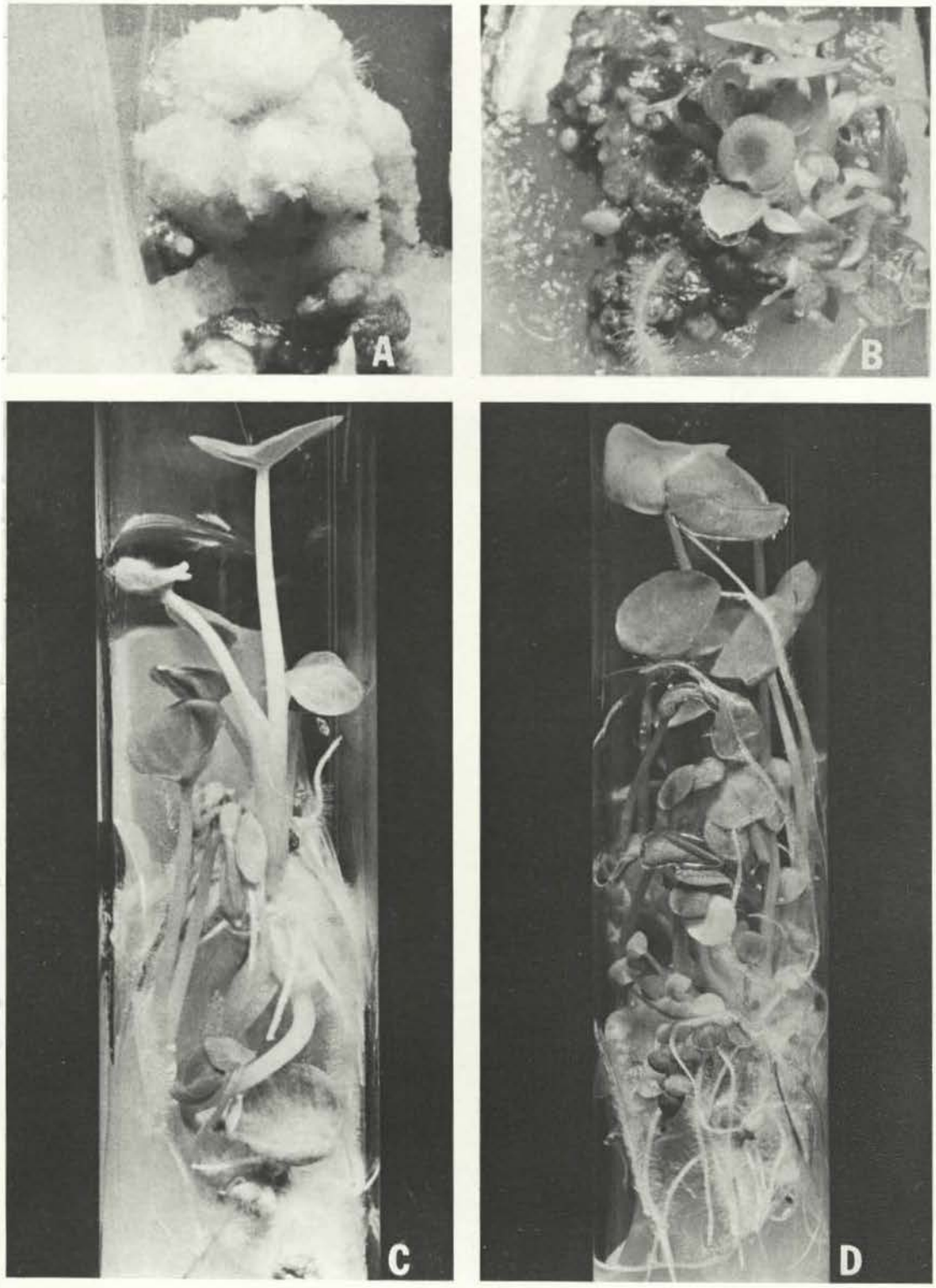

PLATE 1

(*Facing p.* 212)