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Callus formation and plantlet development from axillary buds of taro

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Abstract. Excised lateral buds of taro [*Colocasia esculenta* var. *esculenta* (L.) A.F. Hill] developed into plantlets and formed callus if cultured on media containing taro extract. α-Naphthaleneacetic acid enhanced the process but only if taro extract was also present. The tissue requirements for this variety of taro are different from those of *Colocasia esculenta* var. *antiquorum* (L.) A.F. Hill.

Key words: *Colocasia* – Shoot bud culture

Taro, *Colocasia esculenta* (Araceae, Monocotyledoneae) has always been propagated clonally through small tubers or parts of old ones (de la Pena 1983). This method is slow and tends to spread virus diseases (Ooka 1983). For this reason attempts were and are being made to develop rapid mass clonal propagation procedures which could be used to produce virus-free propagating material. These attempts have been successful with *C. esculenta* var. *antiquorum* (L.) A.F. Hill but not with *C. esculenta* var. *esculenta* (L.) A.F. Hill which includes the important cultivars in the South Pacific. The few procedures developed for these cultivars are inefficient and not easily reproducible (for reviews see Arditti and Strauss 1979; Nyman and Arditti, in press). We have suceeded in culturing axillary-bud explants of *C. esculenta* var. *esculenta* on a modified Murashige and Skoog (1962) medium supplemented with extract of taro corms.

Plants of *C. esculenta* var. *esculenta* cv. Akalomamale from the Solomon Islands cultivated outdoors in Southern California served as explant sources (Fig. 1a). Leaves were cut leaving 3 cm of petiole bases above the corm (15–20 cm high, 8–12 cm diameter, 900–1100 g, four to six leaves). The corms were washed with tap water and household detergent, and rinsed with distilled water before the outermost whorl of the petiole bases was removed. The corm was peeled, approx. 17 cm of tissue was removed from the base, and the remaining portion was washed again as before. It was surface-sterilized by submerging it with constant stirring in household bleach diluted 1:1 (approx. 2.5% NaOCl) containing two

Abbreviations: BA = benzyladenine; CW = coconut water; NAA = α-naphthaleneacetic acid; TE = taro extract

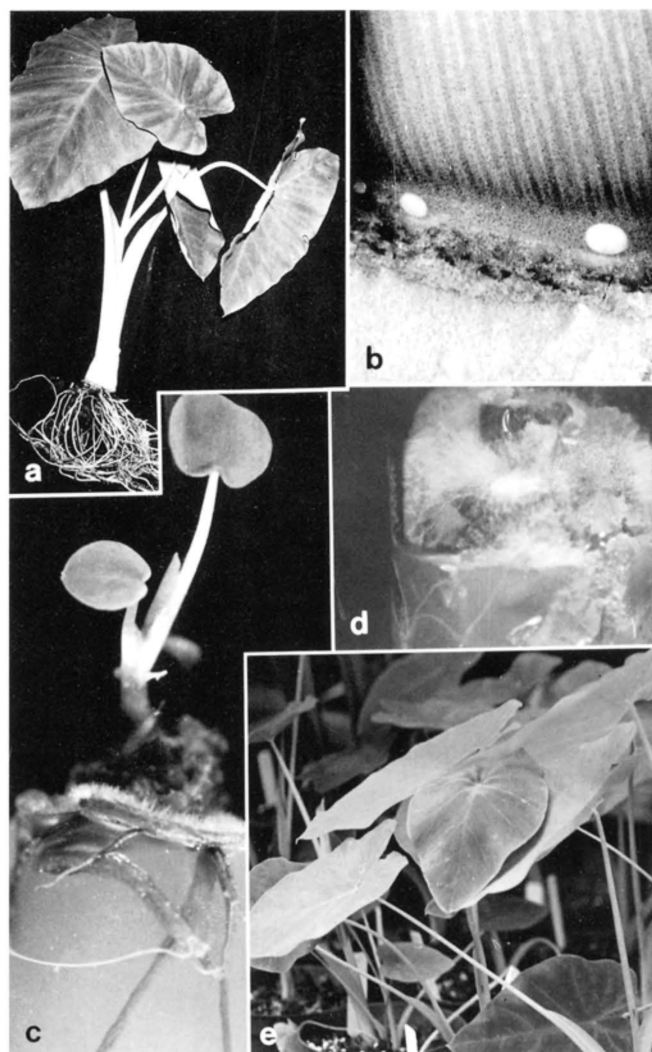

Fig. 1a–e. Axillary-bud culture of taro. **a** Whole plant; ×0.1. **b** Axillary buds in the axil of a leaf that has been removed prior to bud excision (and in this case also for photography); ×6. **c** Plantlet on the second (i.e., hormone-free) medium, one month after the explant was moved from the initial substrate (No. 2). ×2. **d** Callus on a medium containing 2.0 mg NAA, 0.2 mg BA and 20 ml TE·l^{-1} four months after the initiation of culture; ×2. **e** Taro plantlets from excised axillary buds in the greenhouse approx. three months after removal from in-vitro culture; ×0.08

Table 1. Effects of media components on shoot-bud explants of *Colocasia esculenta* var. *esculenta*[a] cultured four months under 16-h photoperiods

No.	Components (initial medium)			Observations		
	Hormones ($mg \cdot l^{-1}$)[b]		Taro extract ($ml \cdot l^{-1}$)	Buds	Callus formation and description[c]	Planlet formation following transfer to the second medium[d]
	NAA	BA				
1	0	0	20	Necrotic	0	0
2	1.0	0	20	Pale green	1; light brown, root forming, growing well	Very good
3	0	1.0	20	Necrotic	0	0
4	0.2	2.0	0	Necrotic	0.3 ± 0.5; light brown, growing poorly	0
5	0.2	2.0	20	Pale green	0.9 ± 0.3; light brown, growing well	0
6	1.0	1.0	20	Necrotic	0.8 ± 0.4; green, growing well, root forming	Good
7	2.0	0.2	20	Pale green	1; light green, growing well, root forming	Very good

[a] Modified Murashige-Skoog medium was used as the basal solution
[b] BA, benzyladenine; NAA, α-naphthaleneacetic acid
[c] Numbers were assigned as follows: no callus = 0, callus formation = 1. These values were used to calculate means and standard deviations. Callus formation, if it occurred, always started after approx. two weeks on the initial medium
[d] Medium consisted of modified Murashige-Skoog solution plus 20 ml $TE \cdot l^{-1}$ and 100 ml $CW \cdot l^{-1}$, but no hormones. Explants were transferred to this medium after two weeks of culture on the initial substrate. Shoots could first be seen after one week of culture on the second medium (three weeks from the start of culture). Plantlet development became apparent two to four weeks after the transfer. All explants on a particular medium responded similarly. Very good: thick petioles, large blades, extensive root system, robust plantlets; Good: slender petioles, small blades, few roots, small plantlets

or three drops of Tween $20 \cdot l^{-1}$ (polyoxyethylenesorbitan monolaurate; Sigma Chemical Co., St. Louis, Mo., USA). The sterilized corms were washed three times with sterile distilled water, petiole bases were removed and buds, 2 mm in diameter or larger (Fig. 1b), as well as shoot tips were excised.

Murashige-Skoog (MS) medium modified by using only one tenth the original microelements level and solidified with 8 $g \cdot l^{-1}$ agar was used as the basal solution to which were added 30 $g \cdot l^{-1}$ sucrose, taro corm extract (TE), coconut water (CW), and hormones (Table 1). The medium, 15 ml, was dispensed into culture tubes, 150 mm long, 22 mm diameter, and autoclaved for 30 min at 120° C and $1.2 \cdot 10^5$ Pa. One bud was cultured in each tube. Treatments were replicated ten times.

The TE was prepared by boiling 600 g of diced (1 cm^3) fresh taro "Akalomamale" corm in 1000 ml distilled water for 5 min and simmering for 1 h. The supernatant was filtered through a Whatman No. 1 filter (Whatman, Maidstone, UK) before being added (20 $ml \cdot l^{-1}$) to the culture medium (Table 1), or stored in a freezer. Coconut water (CW) drained from mature nuts (purchased from local food stores) was filtered through a Whatman No. 1 filter and frozen until use.

Cultures were maintained at $25 \pm 2°$ C on one of several hormone-TE combinations (Table 1). Initially half of the cultures were kept in darkness and the rest under illumination [16 h and 2.5 $mW \cdot cm^{-2}$ provided by 40-W Sylvania Gro-Lux tubes (GTE Products Corporation, Danvers, Mass., USA) and 40-W incandescent lamps]. After two weeks on the intial medium, half of each group of explants was moved to a second substrate, hormone-free but containing CW and TE (Table 1). The other half was transferred to fresh initial medium. Following the transfers all cultures from the light were again placed under illumination and half of those from darkness were moved to light. The other half was kept in darkness until shoots could be seen (approx. a week after the transfer) and then moved to light. All explants (buds, callus, plantlets) were allowed to remain on the second (i.e., hormone-free) medium for 3.5 months. After four months of culture in vitro (two weeks on the initial medium and 3.5 months on the second one) plantlets (6 cm tall with at least four leaves and a well developed root system) were moved to Perlite (Orange County Farm Supply, Orange, Cal., USA) first in a culture room for two to three weeks (one plant per 2-l culture flask) where they were irrigated with 0.5-strength MS medium, and after that in pots (also in Perlite) in the greenhouse with weekly applications of 100 $mg \cdot l^{-1}$ 20:20:20 Peters liquid fertilizer (Peters Fertilizer Products, Fogelville, Penn., USA).

Results were evaluated in terms of explant survival, callus formation, appearance of shoots, root initiation and plantlet development.

Shoots could be seen in those cultures which formed plantlets approximately one week following the transfer (i.e., three weeks after the start of culture). They increased in size and plantlet (Fig. 1c) development became apparent two to four weeks after explants were transferred to the hormone-free (i.e., second) medium following two weeks of culture on initial media containing 1 or 2 $mg \cdot l^{-1}$ α-naphthalene acetic acid (NAA) and not more than 1 $mg \cdot l^{-1}$ N^6-benzylaminopurine (benzyladenine, BA; four to six weeks after the start of culture; Table 1, No. 2, 6, 7). This indicates that the two-week exposure to NAA is necessary for plantlet formation. The different periods required for plantlet formation were affected by the medium and are probably dependent on the physiological or developmental state of the buds at the time they were excised. Illumination during the initial culture period had no effect on plantlet production. Shoot tips failed to form callus or plantlets on any of the media and died within four weeks.

Callus (Fig. 1d) and root formation from axillary buds occurred on NAA concentrations of 0.2–2 $mg \cdot l^{-1}$ and BA levels of 0.2–2 $mg \cdot l^{-1}$ (Table 1). Cytokinins alone (a number of analogs were tested at several concentrations in preliminary experiments) did not induce callus or plantlet formation.

Two major differences between previous culture methods and ours are incorporation of TE in the culture medium, and utilization of axillary buds. High auxin: cytokinin ratios 1) enhance callus initiation, 2) suppress callus growth and development, and 3) stimulate root initiation. Other experiments (data not shown) indicate that CW cannot support growth of *Colocasia esculenta* var. *esculenta* explants in the absence of TE, but enhances plantlet formation and growth when TE is present. Shoot tips (i.e., apical meristems) failed to develop even in the presence of TE.

Cultivars of *Colocasia esculenta* var. *esculenta* are of great importance in the South Pacific where taro is a major staple (Wang 1983). The tissue culture and plantlet production (Fig. 1e) methods described here should prove useful for micropropagation and research.

The fact that *Colocasia esculenta* var. *esculenta* explants do not survive on media which can support those of *Colocasia esculenta* var. *antiquorum* is indicative of physiological differences. These differences coupled with morphological characteristics warrant the establishment of two separate species, *C. esculenta* and *C. antiquorum*.

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