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Induction of callus from axillary buds of taro (*Colocasia esculenta* var. *esculenta*, Araceae) and subsequent plantlet regeneration

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Summary. Axillary buds of taro (*Colocasia esculenta* var. *esculenta*, Araceae) cultured on half strength Murashige-Skoog medium (HMS) containing taro extract (HMSTE) and 2, 4, 5-trichlorophenoxyacetic acid produce a compact, hard, slow growing callus which is not very active morphogenetically and produces only a few plantlets. When cultured on HMSTE plus 5 mg l⁻¹ each of naphthaleneacetic acid and benzyl adenine (HMSNB) the buds produce a fast growing, friable and morphogenetically active callus. Meristematic regions form on the friable callus after 30 days on HMSNB. If transferred to HMSTE at this point the callus gives rise to plantlets. Addition of taro extract to the media is required for the culture of buds, induction of callus and plantlet regeneration.

Key words: axillary buds - callus - *Colocasia esculenta* var. *esculenta* - plant regeneration - taro corm extract

Abbreviations: BA, benzyl adenine; BNA, b-naphthoxyacetic acid; CW, coconut water (liquid endosperm); DW, dry weight; FW, fresh weight; HMS, half strength Murashige-Skoog medium; HMSCW, HMSTE plus 100 ml CW l⁻¹; HMSNB, HMSTE plus 5 mg l⁻¹ each NAA and BA; HMSNB, HMS plus 5 mg l⁻¹ each NAA and BA; HMSNB, HMS plus 25 ml taro extract l⁻¹; HMSTR, HMS plus 2 mg 2,4,5-T l⁻¹; MNA, methyl-1-naphthaleneacetic acid; NAA, naphthaleneacetic acid; OCPAA, ortho-chlorophenoxyacetic acid; TE, taro extract; 2,4,5-T, 2, 4, 5-trichlorophenoxyacetic acid.

**Introduction**

Until recently it was extremely difficult if not impossible to culture explants from taro cultivars belonging to *Colocasia esculenta* var. *esculenta* (Arditti and Strauss, 1979; Nyman and Arditti, in press). These cultivars are of great importance in the South Pacific and continuing efforts to culture them led to the formulation of a method for plantlet production from axillary buds on a medium containing TE (Yam et al., 1990a, 1990b). Incorporation of TE in the medium is a key factor in this procedure which has the shortcomings of limited plantlet and/or callus production. A fast growing and morphogenetically active callus was still needed for both research applications and mass rapid clonal propagation.

**Materials and methods**

**Plant material.** Buds were excised from corms of taro (*Colocasia esculenta* var. *esculenta* Akalomamale) as reported previously (Yam et al., 1990a, 1990b).

**Culture Methods.** Cultures were maintained initially at 25 ± 2° C under 16 h photoperiods of 2.5 mW (40 W Gro-Lux tubes and 40 W incandescent lamps) as reported before (Yam et al., 1990a, 1990b). The buds were cultured in 18 X 145 mm test tubes. Explants were placed on several modifications of HMSTE (Table 1 lists only media which produced callus and/or were used for subcultures and plantlet induction) in the light. Callus was allowed to grow for one month before being sectioned into cubes (ca. 5 mm³; approximately 10 mg fresh weight of each callus; 0.5 mg dry weight for HMSNB and 1.1 mg for HMSTR) and subcultured onto the same medium in Petri dishes (9 cm diameter) in darkness. After four weeks of growth in the Petri dishes the callus masses were cut into 5 mm³ sections and cultured on hormone free HMS (Table 1) under illumination. Plantlets formed in clumps under these conditions and were:

1) separated from each other on reaching a height of 1 cm (ca. one month after they formed on the friable callus and 6-7 months for the compact callus), and 2) moved to HMSCW in 250 ml Erlenmeyer flasks under illumination for ca. two months until they reached a height of six cm and developed four leaves (Table 1). These plantlets were potted in vermiculite and moved to the greenhouse.
Results and Discussion

Very few explants (less than 1%) became contaminated during the course of these experiments. A major reason for this may be the location of the buds under thick, tightly appressed petioles where they were probably free of microorganisms. The surface sterilization procedure (Yam et al., 1990) contributed to the lack of contamination by eliminating potential contaminants from corms and petioles. If not eliminated, these contaminants could, and most probably would, be transferred onto the buds during excision. It is very likely that the detergents and surface sterilants never reached the buds. This may be an important point because exposure to sterilants may have a deleterious effect on some explants (Arditti and Ernst, in press).

Explants on HMSTR (Table 1) became swollen approximately seven days after being placed on the medium. A yellow green somewhat friable callus appeared on the cut end 10 days after that. This callus grew slowly during the subsequent 30 days and became harder and more compact (Fig. 1, 5). Initiation of callus on media lacking TE was extremely limited and inconsistent. When callus did form on such media it grew very slowly. It would seem, therefore, that callus initiation and growth depend on factors(s) present in TE. The limited callus initiation in the absence of TE could be the result of small amounts of the same factor(s) in the explant itself. If so, previous failures in attempts to culture Colocasia esculenta var. esculenta were probably due to the lack of this (these) factor(s).

After being subcultured on HMSTR (Table 1) the callus turned green and more compact. Increases in FW and DW consisted of a small increment (1.5 times) during the first 30 days, a nearly six-fold growth in the second month and slightly more than a doubling in the third month (Fig. 5). The hydration value \[HV = \frac{(FW-DW)}{DW}\] remained constant (Fig. 5). This callus did not become friable at any time, remained slow growing, and produced 2-4 shoots only after it formed small corm-like structures following 6-7 months of culture. The shoots produced few roots on HMS, but many more on HMSCW (Table 1).

A small number of roots was produced by the callus on media containing 1.0, 0.5 or 0.1 mg 2,4,5-T l⁻¹. Root production was related inversely to the concentration of 2,4,5-T (Table 1). The highest number of roots (but still relatively few) was produced on a medium which did not contain this auxin. No roots were produced by the callus or shoots in the presence of 2 mg 2,4,5-T l⁻¹ (Table 1). These observations suggest that in this callus 2,4,5-T functions as an inhibitor of root formation.

Cormlets and subsequently shoots are not produced from the hard callus without 2,4,5-T, but the necessary levels inhibit root formation (Table 1). The borderline between optimal and supraoptimal levels for root formation seems to be 1 mg l⁻¹ (Table 1). Therefore, plantlet production requires the following sequence:

Induction (HMSTR) > subculture (HMSTR) > shoot formation (HMSTR) > root induction (HMSCW).

Buds on HMSNB started to swell one week after being placed in culture and a white friable callus
Table 1. Growth and differentiation of friable and compact callus tissues of *Colocasia esculenta* var. *esculenta*

| Hormone mg 1\(^{-1}\) (medium) | Callus growth | Differentiation | Shoots | Roots | Plantlets | Remarks |
|---------------------------------|---------------|-----------------|--------|------|-----------|---------|
| **2,4,5-T, compact callus** (Fig. 1) |               |                 |        |      |           |         |
| 2 (initial, transfer)           | Slow          | None            | 2-4\(^a\) | None | None      | Shoots bud\(^{-1}\) |
| 1 (transfer)                    | Slow          | None            | Rarely | None | None      |         |
| 0.5 (transfer)                  | Slow          | Occurs          | 3.2±1.4 | None | None      | Roots callus\(^{-1}\) |
| 0.1 (transfer)                  | Slow          | Occurs          | 6.7±2.3 | None | None      | Roots callus\(^{-1}\) |
| 0 (transfer)                    | Slow          | Occurs          | 8.1±1.9 | None | None      | Roots callus\(^{-1}\) |
| 0, **CWB** (transfer)           | Slow          | Occurs          | 13.6±1  | 2-4  | None      | Shoots bud\(^{-1}\), Plaitets bud\(^{-1}\) |

**NAA + BA, friable callus** (Fig. 2, 3)  
NAA, 5+  
BA, 5 (initial)  
No hormones  
0 (transfer)  

|                   |               |                 |        |      |           |
|-------------------|---------------|-----------------|--------|------|-----------|
| **Shoots** develop following 6-7 months of culture and only after the callus has developed cormlets. |               |                 |        |      |           |
| **Shoots** must be transferred to this medium. |               |                 |        |      |           |
| **Buds** (i.e., meristematic regions) were formed on 10-15% of the callus masses. Roots appeared 2 weeks after the callus was transferred to hormone-free medium. Shoots developed one week after that (three weeks after the transfer). |               |                 |        |      |           |
| **The initial callus can be subdivided and subcultured many times resulting in an unlimited number of plants.** |               |                 |        |      |           |

...
which are also present in the flowers of this species. Segments cultured on the same basal medium in the presence of other auxins produces a green, anthocyanin-free callus (Ball, Harborne and Arditti 1972). OCPAA does not induce anthocyanin synthesis because green callus generated on media containing BNA or MNA failed to produce anthocyanins when subcultured onto a OCPAA-containing medium (Ball et al., 1972). OCPAA also failed to induce anthocyanin production in callus tissues of the orchid Epidendrum (Rudolph, Ball and Arditti 1972) and other plants from several families (Ball et al., 1972).

Our present and previous findings suggest that 1) at least at the levels used by us 2,4,5-T is not a suitable auxin for callus induction from taro buds, and 2) a combination of 5 mg l⁻¹ each of NAA and BA is necessary for the induction of a fast growing morphogenetically active callus.

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