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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; HMSTE, HMS plus 25 ml taro extract l^{-1}; HMSTR, HMSTE plus 2 mg 2,4,5-T l^{-1}; MNA, methyl-1-naphthaleneacetate; 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., 1990, in press). 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 el 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 mm^3; 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 mm^3 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.

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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 initiaton and growth depend on factors(s) present in TE. The limited callus initiation in the abscence 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=(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

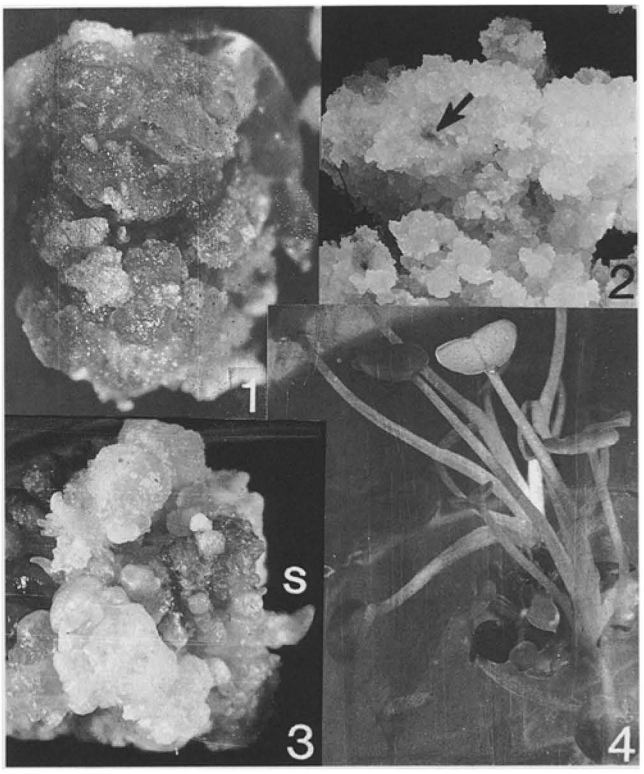

Fig. 1-4. Callus formation and plantlet regeneration in taro. 1. Compact callus produced on HMSTR. X 3.5. 2. Friable callus produced on HMSNB with a newly formed meristematic area (arrow). X 2. 3. Young shoot (s) emerging from friable callus. x 4. 4. Rooted (r) plantlets in flask. X 2.5.

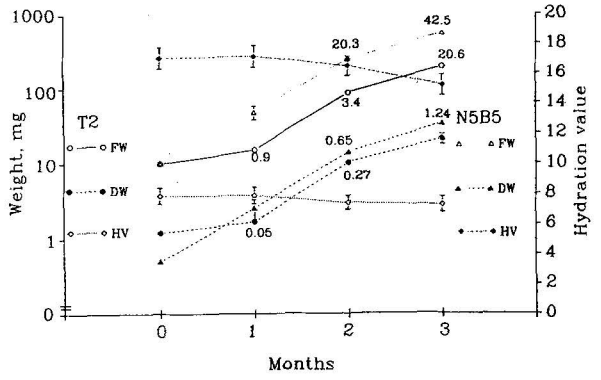

Fig. 5. Weight increases and hydration value [(FW-DW)/DW] of compact (T2) and friable (N5B5) callus.

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 l^{-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	None	Rarely	None	
0.5 (tranfer)	Slow	Occurs	None	3.2±1.4	None	Roots $callus^{-1}$
0.1 (transfer)	Slow	Occurs	None	6.7±2.3	None	Roots $callus^{-1}$
0 (transfer)	Slow	Occurs	None	8.1±1.9	None	Roots $callus^{-1}$
0, CW[b] (transfer)	Slow	Occurs	None	13.6±1	2-4	Roots $shoot^{-1}$; Platlets bud^{-1}
NAA+BA, friable callus (Fig. 2, 3)						
NAA, 5+ BA, 5 (initial)	Fast	None	None[c]	None	None	
No hormones 0 (transfer)	Stops	Occurs	6.1±1.9[d]	7.9±1.5[d]	Yes	Shoots, roots $callus^{-1}$

[a]Shoots develop following 6-7 months of culture and only after the callus has developed cormlets.
[b]Shoots must be transferred to this medium.
[c]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).
[d]The initial callus can be subdivided and subcultured many times resulting in an unlimited number of plants.

appeared at their bases 7-14 days after that (a total of 2-3 weeks after excision). This callus (Fig. 2) grew rapidly reaching a diameter of 1 cm and a height of 6-7 mm within a month. Following subculture on HMSNB the FW and DW increased five-fold during the first month and 20-30 times in two months (Fig. 5). The hydration value was constant, but 2.3 times that of the compact callus (Fig. 1). This difference is a reflection of the friable nature of the tissue.

The friable callus masses were white and translucent during the first month, but turned greyish-white and opaque after that (Fig. 2). After 30 days in culture 10-15% of the friable callus masses formed meristematic regions (Fig. 2; Table 1). If the callus was allowed to remain on HMSNB the meristematic regions increased in number but failed to produce shoots. In few cases shoots were produced, but did not proliferate. However, if the callus was transferred onto HMSTE the meristematic regions formed shoots (Fig. 3) and plantlets (Fig. 4; Table 1).

Multiple (about 10 per 5 mm diameter callus section) green shoots formed on the hormone-free medium (HMSTE, Table 1) within 2-4 weeks. These shoots developed 2-3 leaves (2-3 mm wide and 5 mm long) and many long, slender roots after reaching a height of 5-10 mm (2-3 weeks post induction). On being moved to HMSCW the plantlets developed additional roots and leaves and grew reaching a height of 5 cm after two months. Their roots were very long, branching and slender. Leaves were 3-4 in number with dark green blades, 10-15 mm in diameter and greenish-white, slender petioles. After being potted in vermiculite and moved to the greenhouse these plantlets grew well and developed normally.

The callus which formed on HMSTR was friable at first but became hard and compact under continued culture. It did not become friable again after being cultured on HMSNB. Callus generated on HMSNB was friable from the outset and did not become hard and compact on HMSTR. The only difference between HMSTR and HMSBN is in their known hormone content (the hormonal content of TE itself is unknown). HMSTR contains an auxin (2,4,5-T), but no cytokinin because previous work showed that root formation occurs when both of these hormones are present in a medium. To maintain an undifferentiated callus BA had to be excluded. HMSNB contains both an auxin (NAA) and and a cytokinin (BA). Therefore it seems that 1) HMSTR selects for a compact, slow growing callus which is not very active morphogenetically, 2) HMSNB selects for a friable, fast growing, morphogenetically active callus, and 3) neither of the two media can change the nature of an existing callus. The latter is an indication that the selection during culture is for a specific cell type. Whether this will have any bearing on crop plants produced from each callus is not clear at present.

Selection for different callus types by auxins has been reported previously. Callus derived from stem segments of *Dimorphotheca sinuata* cultured on a medium containing *O*CPAA produced delphinidin-3-glucoside and cyanidin-3-glucoside, two anthocaynins

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). *O*CPAA does not induce anthocyanin synthesis because green callus generated on media containing BNA or MNA failed to produce anthocyanins when subcultured onto a *O*CPAA-containing medium (Ball et al., 1972). *O*CPAA 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.

Acknowledgments

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