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Plant regeneration in vitro of South Pacific taro (*Colocasia esculenta* var. *esculenta* cv. Akalomamale, Aracea)

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Summary. Axillary bud explants from South Pacific (Solomon Islands) taro, *Colocasia esculenta* var. *esculenta* cv. Akalomamale (Araceae) cultured on a modified Murashige-Skoog medium containing 1 mg NAA l^{-1} and TE formed callus and produced multiple plantlets. Explants died if NAA was present at levels lower than 0.1 mg l^{-1}. BA was not required and may have been inhibitory. Plantlets developed faster and became larger following transfer to a hormone-free medium two weeks after the start of culture. Fully grown plants were established in a potting mix and are growing well in a greenhouse.

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

Abbreviations: BA, benzyladenine; BM, basal medium; Ca, Colocasia esculenta var. antiquorum; Ce, Colocasia esculenta var. esculenta; Ck, cytokinin(s); CW, coconut water; HSMSM, half strength Murashige Skoog macroelements; HSMS, half strength Murashige and Skoog medium; IM, initial medium(ia); MS, Murashige and Skoog medium; NAA, naphthaleneacetic acid; SM, second medium; TE taro corm extract; UCI, University of California, Irvine.

Introduction

Taro, *Colocasia esculenta*, one of the earliest food plants used by humans, is as important in the South Pacific as grains are in other parts of the world (Wang 1983). Two virus diseases, Alomae and Bobone, threaten its very existence. Dasheen mosaic virus is also a problem (Johnston 1960, Gollifer and Brown 1972, James et al. 1973, Kenten and Woods 1973, Ooka 1983). Use of the upper portions of corms for propagation (de la Pena 1983) spreads these and other systemic diseases. To reduce or eliminate this problem it is necessary to develop methods for the production of disease free propagation material (Arditti and Strauss 1979).

Systemic infections, including viruses, can be eliminated through tissue culture of small explants. Unfortunately, however, most existing procedures for tissue culture of taro were developed with Ca and are not suited for those belonging to Ce. The few procedures developed for Ce are inefficient and not easily reproducible (Mapes and Cable 1972, Jackson et al. 1977, Arditti and Strauss 1979, Nyman and Arditti in press). This is a major problem since the important cultivars in the South Pacific belong to *Ce.* The procedure described here, a modification of an earlier method (Yam et al. 1990), is for Ce cultivars.

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Materials and methods

Plant material. Plants of Ce Akalomamale collected in the Solomon Islands, propagated in a greenhouse and grown outdoors at UCI, served as explant sources. Corms (about 1 kg, 20 cm high and ca. 10 cm in diameter) were harvested in February 1989 and their leaves were removed by cutting the petioles 30 mm above the corm. Following surface sterilization, radial cuts, 1 cm apart, were made into the outermost layer of petiole bases and the segments were removed to expose the axillary buds below them.

Circular cuts were made around axillary buds with a pointed scalpel blade leaving 0.5-1.0 mm of tissue around each bud. Following removal of all buds from one whorl, explants were excised from other whorls in a similar manner. Axillary buds are large enough to be seen with the naked eye. Apical meristems were removed under a dissecting microscope. To test for sterility explants were placed on 10 ml initial medium in plastic Petri dishes (9 cm diameter) for 3 days.

Surface sterilization. Following removal of the outermost whorl of petioles corms were scrubbed with running water and household detergent (Lux brand). After tissue from the base was cut away, corms were washed with distilled water, submerged in sterilant (Clorox:water, 1:1, v:v containing 2 drops Tween 20 l^{-1}) for 30 min with stirring, and rinsed three times with sterile distilled water.

Culture Conditions. Cultures and plantlets in Perlite-containing flasks were maintained under 16 h days, a light intensity of 2.5 mW cm^{-2} (provided by 40 W Gro Lux fluorescent tubes and 25 W incandscent lamps) at 25±4° C. Potted plants in the greenhouse were grown under natural light and photoperiods at 24±° C during the day and 20±4° C at night.

Culture Media. MS modified by adding: microelements and niacin at one tenth the original concentration, 20 mg glycine l^{-1}, 0.5 mg thiamine l^{-1}, and 20 ml TE l^{-1} served as BM. For IM 0-5 mg

NAA l^{-1} and/or 0-5 mg BA l^{-1} were added to BM. SM was prepared by adding only 100 ml CW l^{-1} to BM.

TE was prepared by dicing (1 cm^3 cubes) 600 g of freshly harvested corms of Ce Akalomamale, boiling for 5 min in 1000 ml distilled water, and simmering for 1 h. The supernatant, filtered through a Whatman No. 1 filter, was used immediately or frozen for future use.

CW drained from mature nuts purchased in local food stores was filtered through a Whatman No. 1 filter and either added to a culture medium or frozen.

Perlite (Orange County Farm Supply, Orange, CA) moistened with HSMSM in two liter Erlenmeyer flasks was used as a substrate for larger plantlets (at least 6 cm tall). HSMSM was also used to irrigate these plantlets.

All culture media and the Perlite, but not mixtures and fertilizers used in the greenhouse, were sterilized by autoclaving. Media and treatments were replicated ten times.

Cultivation of plantlets. Plantlets, either after 2-3 wk in flasks on Perlite or taken directly from culture vessels (if they were robust), were moved to the greenhouse, potted in Perlite and repotted every 4-6 wks. After approximately six months the larger plantlets were moved to a mixture of Perlite and fir bark. Potted plantlets were fertilized weekly with 100 mg l^{-1} of Peters Professional Water Soluble Fertilizer M-77, Exclusive Chelating Formula (Peters Fertilizer Products, Fogelsville, PA 18051).

Procedure. Buds were cultured for two weeks on one of 16 IM in the light (Fig. 1-3). After that half of the explants were moved to a SM (Fig. 4-6). The other half was transferred to fresh IM (Fig. 1-3). In both cases callus or plantlets were cultured for an additional 12 wks (Fig. 1-6). Plantlets which formed on IM were moved to SM for further development. On reaching a height of 6 cm with well developed root systems and at least four leaves plantlets were moved to Perlite.

Evaluation of results. Explant survival, callus development, appearance of shoots and/or roots and plantlet production were recorded. To allow for statistical analysis bud survival, callus development, root formation and plantlet regeneration were each assigned a value of 1 whereas their absence was designated as 0.

Results and Discussion

None of the cultures became contaminated and all buds were alive at the end of three wks. Buds on media without NAA died by the 6th wk (Fig. 1) . Those on low-NAA media were dead after 10-15 wks (Fig. 1, 2).

Only buds on media containing 1 mg NAA l^{-1} or more survived. BA concentrations did not seem to have any effects except perhaps to delay by one wk the death of buds on low NAA media.

Callus formation occurred on several media during the initial three wks (Fig. 2). Tissues on low NAA substrates started to die after six wks and all were dead at the end of the experiment (Fig. 2). Roots appeared first on media containing no BA or low levels of this Ck (i.e., high NAA:BA ratios). Additional roots appeared 6, 10 and 15 wks after the start of the experiment (Fig. 3). Leaves formed following the appearance of roots after a total of 15 wks of culture on media which contained 1 or 5 mg l^{-1} NAA. Ck levels had minimal or no effects (Fig. 3).

All buds were still alive one wk after being transferred to SM (Fig. 4). With one exception (buds moved from a medium containing 0.1 mg NAA l^{-1} and no BA), explants from low NAA media died within four wks of transfer (Fig. 4). Some buds from high NAA media also died (Fig. 4). Highest survival was by buds from media containing 0.1, 1 or 5 mg NAA l^{-1} and no BA.

Buds from NAA-free media did not form callus on SM (Fig. 5). Callus was formed by all buds from media containing 1 or 5 mg NAA l^{-1} and two of those with 0.1 mg NAA l^{-1} (Fig. 5). Tissues formed by buds from low NAA media started to die before those from high auxin substrate (Fig. 5).

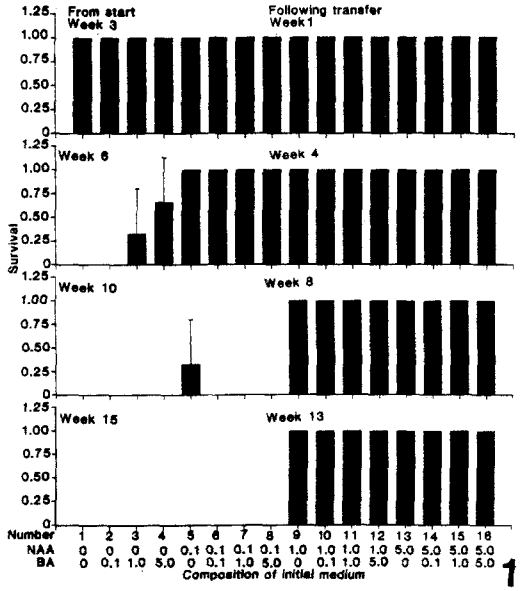
Roots appeared first on buds and tissues from media which contained no BA or only 0.1 mg l^{-1} of this Ck (Fig. 6). Subsequent root formation was on tissues from several NAA and BA concentrations (Fig. 6, 7). Leaves were first seen four wks after transfer from media containing 0.1 mg NAA l^{-1} and no BA or 1 mg NAA l^{-1} plus 0.1 mg BA l^{-1}. Subsequent leaf formation occurred on tissues from several media (Fig. 6, 7).

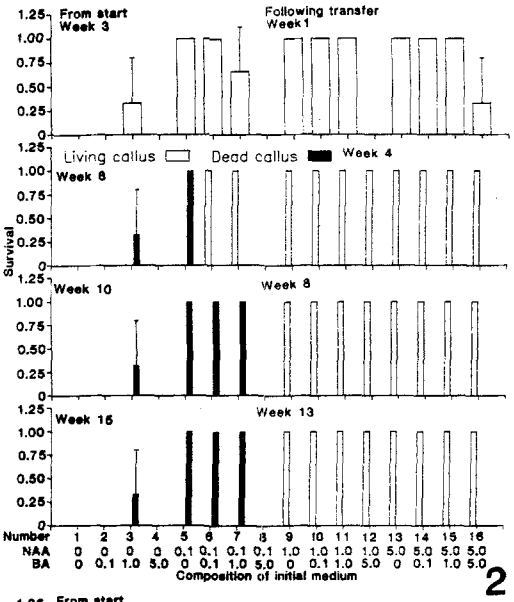
Plantlets on the hormone-free medium were taller, darker green in color and had more and longer roots (Table 1). The number of leaves was the same on both media (Table 1). Plantlet production occurred both in the presence and absence of NAA and BA, but it was more rapid on hormone-free media (Fig. 3 vs Fig. 6). Buds which were not moved from IM produced 5-6 plantlets each. Plantlet yield per bud can be increased considerably by dividing and subculturing the callus which is produced by the buds. All potted plants in the greenhouse survived and are growing normally (Fig. 8).

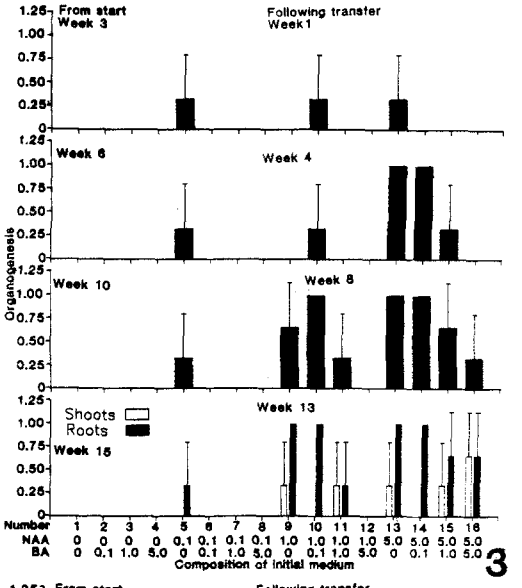
Table 1. Characteristics of South Pacific taro cv. Akalomamale plantlets regenerated in vitro from axillary bud explants.

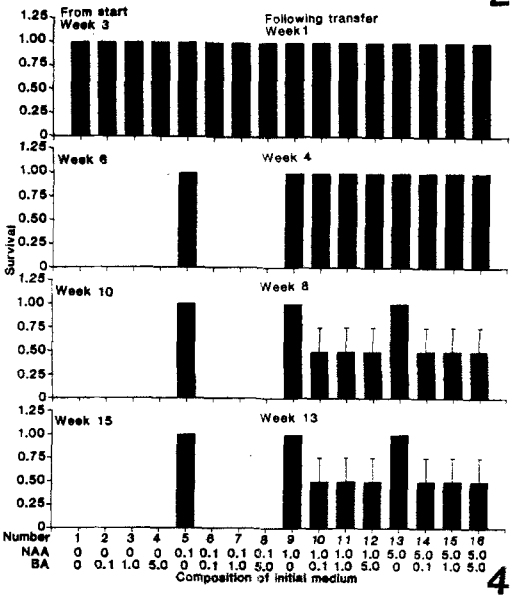
Characteristic		Medium: Initial[a]	Medium: Second[b]
Leaf	number	5.0±0.6	64.2±0.8
Blade	color[c]	Lt gr	Gr
	width[d]	4.6±2	14.7±7
	length[e]	5.1±1.8	16.6±5.4
Petiole	length, cm	1.0±0.6	8.8±3.2
	color	Lt gr	Lt gr
Root	length, cm	2.9±1.6	6.6±2.1
	color	Lt br-lt gr	Lt br-gr
Plant	height, cm	1.5±0.5	11.5±2.2

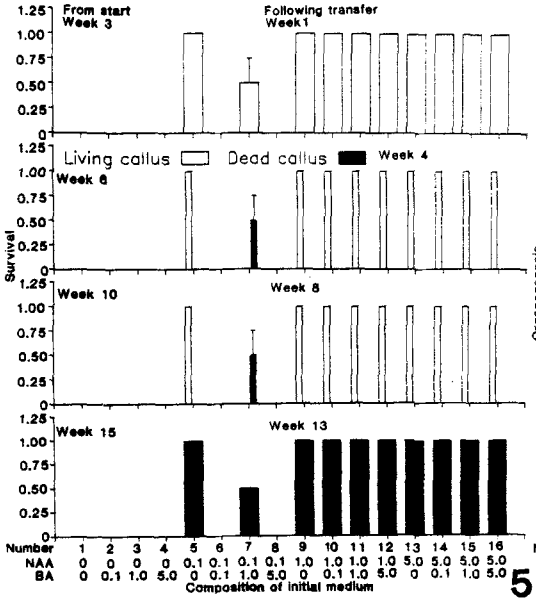
[a]Contains NAA and/or BA and TE (see text).
[b]Hormone-free, contains TE and CW.
[c]Br, brown; gr, green; lt, light.
[d]Measured at widest part, mm.
[e]Measured at longest part, cm.


From start Week 3
Following transfer Week1
Week 6
Week 4
Week 10
Week 8
Week 15
Week 13
Survival
Number 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
NAA 0 0 0 0 0.1 0.1 0.1 0.1 1.0 1.0 1.0 1.0 5.0 5.0 5.0 5.0
BA 0 0.1 1.0 5.0 0 0.1 1.0 5.0 0 0.1 1.0 5.0 0 0.1 1.0 5.0
Composition of initial medium
1


From start Week 3
Following transfer Week1
Living callus
Dead callus
Week 4
Week 6
Week 10
Week 8
Week 15
Week 13
Survival
Number 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
NAA 0 0 0 0 0.1 0.1 0.1 0.1 1.0 1.0 1.0 1.0 5.0 5.0 5.0 5.0
BA 0 0.1 1.0 5.0 0 0.1 1.0 5.0 0 0.1 1.0 5.0 0 0.1 1.0 5.0
Composition of initial medium
2


From start Week 3
Following transfer Week1
Week 6
Week 4
Week 10
Week 8
Shoots
Roots
Week 15
Week 13
Organogenesis
Number 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
NAA 0 0 0 0 0.1 0.1 0.1 0.1 1.0 1.0 1.0 1.0 5.0 5.0 5.0 5.0
BA 0 0.1 1.0 5.0 0 0.1 1.0 5.0 0 0.1 1.0 5.0 0 0.1 1.0 5.0
Composition of initial medium
3


From start Week 3
Following transfer Week1
Week 6
Week 4
Survival
Week 10
Week 8
Week 15
Week 13
Number 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
NAA 0 0 0 0 0.1 0.1 0.1 0.1 1.0 1.0 1.0 1.0 5.0 5.0 5.0 5.0
BA 0 0.1 1.0 5.0 0 0.1 1.0 5.0 0 0.1 1.0 5.0 0 0.1 1.0 5.0
Composition of initial medium
4


From start Week 3
Following transfer Week1
Living callus
Dead callus
Week 4
Week 6
Survival
Week 10
Week 8
Week 15
Week 13
Number 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
NAA 0 0 0 0 0.1 0.1 0.1 0.1 1.0 1.0 1.0 1.0 5.0 5.0 5.0 5.0
BA 0 0.1 1.0 5.0 0 0.1 1.0 5.0 0 0.1 1.0 5.0 0 0.1 1.0 5.0
Composition of initial medium
5

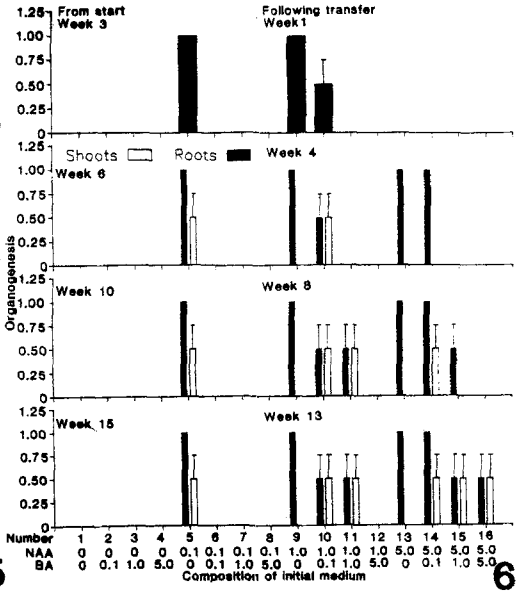

From start Week 3
Following transfer Week1
Shoots
Roots
Week 4
Week 6
Organogenesis
Week 10
Week 8
Week 15
Week 13
Number 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
NAA 0 0 0 0 0.1 0.1 0.1 0.1 1.0 1.0 1.0 1.0 5.0 5.0 5.0 5.0
BA 0 0.1 1.0 5.0 0 0.1 1.0 5.0 0 0.1 1.0 5.0 0 0.1 1.0 5.0
Composition of initial medium
6

Fig. 1-6. In vitro culture of South Pacific taro. 1. Survival of bud explants. 2. Callus production and survival. 3. Shoot and root production. 4. Survival of bud explants following transfer to a medium containing taro extract and coconut water but no hormones. 5. Callus development and survival following transfer to the taro extract and coconut water medium. 6. Shoot and root production following transfer to the hormone free medium.

Attempts to culture shoot tips or other explants of Ce have not been successful on media which can support those of Ca (Arditti and Strauss 1979). This suggests fundamental physiological differences between the apical meristems and axillary buds of Ca and Ce. The reasons for the different requirements are not clear, but it is intriguing to note that Ce can be cultured only in the presence of TE (Yam et al. 1990).

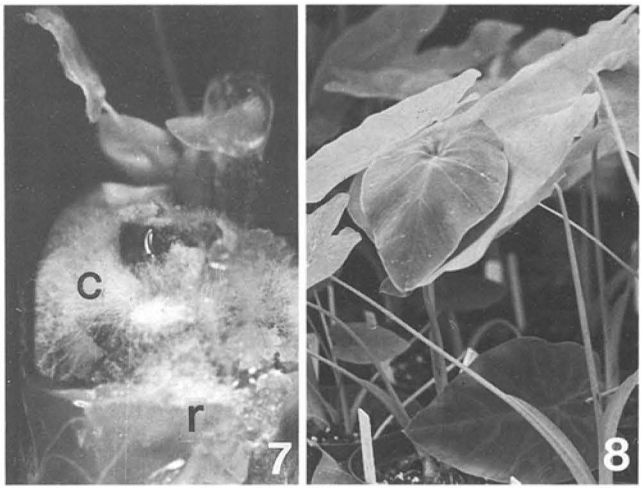


Fig. 7-8. Plantlet regeneration in South Pacific taro. 7. Roots and leaves arising from a callus mass. X 2.7. 8. Plants in the greenhouse. X 0.08. Explanation of symbols: c, callus; r, roots.

Potato extract, a beneficial media additive for the culture of wheat anthers (Chuang et al. 1978) and other grasses had the same effect on taro explants as TE (unpublished). It is interesting to note that potato is a dicotyledonous plant whereas taro is monocotyledonous, and in both cases the extract is from a storage organ.

BM contains appropriate levels of minerals and sucrose. Therefore it is not likely that TE acts by raising the concentrations of these substances to optimal levels. Analyses of taro tubers show that they contain at least 19 of the essential amino acids, a number of vitamins, several pigments, as well as some fatty acids, sterols and alkaloids (Sunnell and Arditti, 1983). Any of these or other tuber components singly and/or in combinations could be the active principle(s).

Plants of Ca UCI Runner produced through tissue culture developed normally in the greenhouse, were disease-free, and grow well in the field (Arditti and Strauss 1979; Nyman and Arditti in press). The same should be true for regenerants of Ce Akalomamale and other cultivars. Therefore the method we describe here should prove useful for research, in vitro selection and practical applications.

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