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A Simple and Efficient Protocol for Rapid Regeneration and Propagation of Taro (*Colocasia esculenta* (L.) Schott.) *in Vitro* from Apical Meristems

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ABSTRACT

An efficient and simple protocol was developed for the *in vitro* regeneration and propagation of taro (*Colocasia esculenta*) cv. 'Muktakeshi'. Apical meristems (~1 cm) excised from leaf blight-resistant taro cultivar cv. 'Muktakeshi' grown in a net house were used as explants. Most multiple shoots (i.e., 3.6/explant), which formed on Murashige and Skoog (MS) medium supplemented with 5 mg L^{-1} 6-benzyladenine and 1 mg L^{-1} α-naphthaleneacetic acid, could be rooted by transferring the 4-week-old shoots to MS basal medium without plant growth regulators. After 2 weeks, well developed plantlets were hardened in plastic cups in potting mixture (vermiculite + sand, 1:1, v/v). Acclimatized plants were transferred to 30-cm plastic pots containing top soil and vermicompost (3:1, v/v) where they grew well.

Keywords: tissue culture, 6-BA, α-NAA, multiple shoots, root induction

INTRODUCTION

Taro (*Colocasia esculenta* (L.) Schott.), a member of the Araceae family, is a traditional root crop of the tropics grown for its edible corms and leaves, and is believed to be one of the earliest cultivated root crops in the world (Plucknett 1976; Kuruvilla and Singh 1981).

The corms, leaves and petioles are used as a vegetable. The taro plant is a rich source of carbohydrates, proteins, minerals and vitamins and has medicinal properties to reduce tuberculosis, ulcers, pulmonary congestion and fungal infection (Brown *et al*. 2004; Sharma *et al*. 2008; Singh *et al*. 2012). Besides the medicinal properties, taro corms are utilized in various industries for the preparation of high fructose syrup and alcohols (Misra *et al*. 2008). Taro is predominantly vegetatively propagated (Strauss *et al*. 1979; Ivancic 1992) and its relatively low propagation ratio has become a bottleneck in large-scale cultivation of this crop (Zhou *et al*. 1999). Apart from this, leaf blight caused by *Phytophthora colocasiae* and viral diseases are globally the major constraints in taro production (Misra *et al*. 2008). These pathogens, especially viruses, are carried through infected corms over time and place (Babu *et al*. 2010, 2011).

In vitro techniques offer an alternative, reliable and rapid method for the production of high-quality, disease-free planting material. Several researchers have reported *in vitro* propagation of taro in various cultivars using different combinations of plant growth regulators (**Table 1**). Micropropagation of taro has been reported through protocorm-like bodies (Sabapathy and Nair 1992) and callus culture (Gupta 1985; Yam *et al*. 1990). Chand *et al*. (1999) reported a two-stage micropropagation protocol for taro using a sterile potting mixture at the first stage and thereafter inoculating meristems on a modified Murashige and Skoog (MS; 1962) medium supplemented with TDZ. Though there are reports of successful micropropagation of taro, few are used for commercial purposes due to their lengthy and cumbersome procedures. Several of the protocols involve 3-4 media changes for achieving the target (Tuia 1997; Chand *et al*. 1999) and use costly chemicals such as polyamines (Sabapathy and Nair 1992). Therefore, an attempt was made to simplify the *in vitro* propagation protocol. The Central

Table 1 Reports of *in vitro* propagation of taro (*Colocasia esculenta* var. *esculenta*).

Cultivar	Explant used	Medium and culture conditions	Hormone combination	Reference
Akalomamale	Axillary buds	Modified Murashige and Skoog (1962) medium, 16 h days at 25±4°C	1 mg L^{-1} NAA	Yam *et al*. 1990
Dotare	Apical shoots	Modified Murashige and Skoog (1962) medium	1 mg L^{-1} NAA and BA	Malamug *et al*. 1992
Keladi Birah	primary shoot apices	Modified Linsmaier and Skoog (1965) medium	5.5 mg L^{-1} NAA and 0.2 mg L^{-1} kinetin or 1.85 mg L^{-1} NAA and 2 mg L^{-1} kinetin	Sabapathy and Nair 1992
Niue	Meristem	Murashige and Skoog	TDZ 0.6 mg L^{-1}	Chand *et al*. 1999
White Dasheen	Meristem domes	Murashige and Skoog (1962) medium, 25 ± 2°C; 12-h photoperiod	5 mg L^{-1} BA, 1 mg L^{-1} IAA for shoot induction; 0.25 mg L^{-1} NAA, 1.5 g/l charcoal for root induction	Ko *et al*. 2008
Not mentioned	Shoot meristem	Murashige and Skoog (1962) medium	5 µM IAA and 7.5 µM BAP for shoot induction; 2 µM IAA for root induction	Verma and Cho 2010

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Short Communication

Tuber Crops Research Institute (CTCRI) has released many high yielding cultivars of taro and cv. 'Muktakeshi' is a leaf blight-resistant variety. Rapid multiplication of these varieties on a commercial scale is essential to supply disease-free, good quality planting material to farmers.

Here, we report a simple multiplication protocol for taro cv. 'Muktakeshi' for possible commercial level multiplication of taro.

MATERIALS AND METHODS

All chemicals were purchased from Fisher Scientific (Mumbai, India) and HiMedia (Mumbai, India).

Taro (leaf blight-resistant cv. 'Muktakeshi', released by the CTCRI, India) corms were dispersed in moist sand in aluminium trays to facilitate sprouting in a net house. Once the apical meristem grew to approximately 1 cm they were excised from the tuber and surface sterilized as follows. The excised tips were washed in running tap water for 2 h then suspended in 2% (v/v) mild detergent solution (Labolene) for 1 h with frequent agitation at 50 rpm. Explants were then washed in tap water to remove detergent, rinsed twice in distilled water, then surface sterilized in 70% ethyl alcohol for 1 min. Outer layers of the explants were removed, trimmed and re–disinfested in 0.1% mercuric chloride containing a few drops of Tween-20 for 3 min and rinsed 4-5 times with autoclaved double distilled water and blotted dry on sterile Whatman No.1 filter paper.

Explants were cultured on MS (Murashige and Skoog 1962) basal medium containing 30 g L^{-1} sucrose and 7 g L^{-1} agar supplemented with various concentrations of 6-benzyladenine (BA) and α-naphthaleneacetic acid (NAA) (**Table 2**). The pH of the medium was adjusted to 5.8 prior to autoclaving. Explants were placed vertically with the basal cut surface in contact with the medium and incubated at 25 ± 2°C in a 12-h photoperiod and a photon flux density of 24 µmoles photons m^{-2} s^{-1}.

The 4-weeks-old regenerated shoots were aseptically transferred and cultured on MS basal medium with 30 g L^{-1} sucrose for root induction.

For hardening, well developed plantlets were removed carefully from the medium, washed with sterile water to remove the traces of agar and finally dipped in a fungicide (Carbendezim 1%, BASF India Ltd., Mumbai, India). The plantlets were then planted in disposable plastic cups (6 cm) containing potting mixture (vermiculite + sand, 1:1) and transferred to a growth chamber (Sanyo Versatile Enviromental Chamber, MLR-351H, Japan). The plantlets were initially covered with polythene bags for one week to maintain high humidity. Thereafter they were monitored daily for growth and those that grew were further transferred to a greenhouse to 30-cm plastic pots containing top soil enriched with vermicompost (3:1). The percentage survival was recorded one month after acclimatization while the phenotype of surviving plants was visually assessed after 2 months using the equation:

Plantlet survival (%) = (No. of surviving plantlets / Total No. plantlets) × 100.

Each treatment contained 9 replicates with a single explant/test tube. Data recorded after 4 weeks was analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test for comparison of significance among mean values (P = 0.05). All analyses were performed using SAS for Windows v. 9.2 (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

For rapid *in vitro* propagation of taro, shoot meristem was cultured on MS fortified with 6-BA and α-NAA at combinations and concentrations given in **Table 2**. In all the combinations used, the concentration of 6-BA to α-NAA was at a ratio of 5: 1. All trials gave positive response though the shoot length and the number of shoots produced depended on the concentration of 6-BA and α-NAA. Malamug *et al*. 1992 has also shown the efficiency of shoot induction in taro by using 6-BA and α-NAA combination (1:1 ratio), where they also observed rooting. However, Yam *et al*. 1990 reported 6-BA to be toxic in formation of callus (cv. 'Akalomamale') and subsequent generation of multiple shoots. In the present study, at very low and high concentrations, there was a significant reduction in the shoot length as well as number of shoots initiated. At lower concentrations the shoot proliferation was less probably due to not meeting the threshold level of growth regulators required to induce the shoot and at higher concentrations the growth retardation could be due to the toxic effect reported by Yam *et al*. 1990. In our study, highest number of shoot bud initiation and proliferation of micro-shoots was observed in medium supplemented with 5.0 mgl^{-1} 6-BA and 1.0 mgl^{-1} α-NAA. Within 10 days of culture, shoot buds started appearing and each explant produced an average of

Table 2 Effect of 6-BA and NAA on shoot initiation and formation of multiple shoots from apical meristem explants of taro (*Colocasia esculenta* var. *esculenta*) cv. 'Muktakeshi'.

Plant growth regulators (mg/l)		Shoot length after 4 weeks (cm)	Multiple shoot after 4 weeks (cm)
6-BA	NAA		
1	0.2	2.93 a	2.22 cd
2	0.4	2.65 bc	2.44 bc
3	0.6	2.52 cd	1.88 d
4	0.8	2.47 d	1.77 d
5	1	2.75 b	3.66 a
6	1.2	2.52 cd	2.77 b
7	1.4	2.24 e	2.22 cd
8	1.6	1.66 f	1.22 e

Values are means of 9 replicates. Means within a column with the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

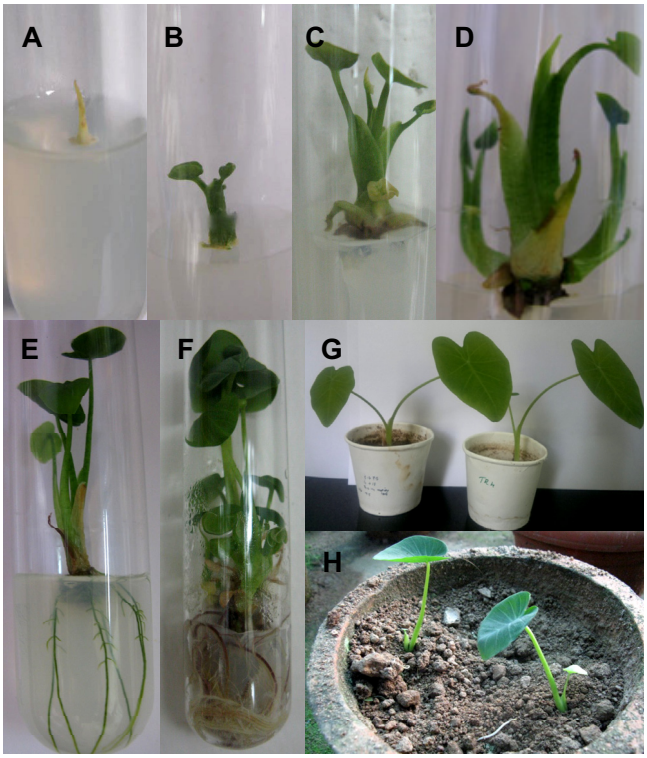

Fig. 1 Micropropagation of taro (*Colocasia esculenta*) cv. 'Muktakeshi' from apical meristems. (**A**) Apical meristems are inoculated into shoot development medium. After 1 week of culture the explants turn green. (**B**) Shoot development is initiated with the formation of young leaves. Further incubation results in (**C**) elongation of the shoots with more leaves and (**D**) the formation of multiple shoots after 3 weeks of culture. The shoots are aseptically transferred to root developing medium where (**E**) they form adventitious roots and (**F**) develop into mature plantlets. Fully matured plantlets are transferred to (**G**) plastic cups with potting medium containing vermiculite and sand (1:1, v/v), and acclimatized plantlets are placed in (**H**) pots enriched with top soil and vermicompost (3:1, v/v) in a glasshouse.

3-4 completely developed shoots within 4 weeks (**Table 2, Fig. 1**). Contradictory to our results, Ko *et al.* (2008) have reported maximum number of shoots in case of white dasheen (*Colocasia esculenta*) on medium containing high concentration of 6-BA (8.0 mg l^{-1}) along with 3.0 mg l^{-1} IAA. We observed that at 8.0 mg l^{-1} 6-BA resulted in yellowing of micro-shoots.

For rooting experiments shoots of length not less than 3-4cm were transferred to MS basal medium. Within 7 days of incubation 100% rooting was observed (**Fig. 1**). Verma and Cho (2010) could achieve 100% rooting only on medium augmented with IAA (2 μM). In our experiments, roots developed on MS basal medium were healthy, branched and were of approximately 8-10 cm; hence other growth regulators were not used for rooting experiments. These results show that shoot induction, proliferation, and rooting are genotype dependent.

Young plantlets potted in vermiculite sand mixture (1:1 ratio) were transferred to growth chamber where they produced new leaves within 7 to 10 days. All the plantlets acclimatized easily under growth chamber conditions, showed great vigor and established themselves. Acclimatized plantlets were transferred to greenhouse under 70% shade in plastic pots (30 cm wide) containing top soil enriched with vermicompost at 3:1 ratio. Ninety two to 95% of the plantlets survived in the pot mixture.

The present study demonstrates a simple and highly efficient protocol for initiation of multiple shoots and roots from apical meristem explants of taro cv. 'Muktakeshi'. The protocol can be applied for commercial mass production and distribution of cv. 'Muktakeshi' to farmers which is crucial to reduce yield loss caused by leaf blight disease. Although, the present study has been conducted in cv. 'Muktakeshi', further refinements by including more cultivars of taro may render this as a universal protocol which could be the next step of this research.

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