



RAPID *IN VITRO* PROPAGATION OF WESTERN GHATS CULTIVAR - *COLOCASIA ESCULENTA* FOR THE HIGH FREQUENCY OF PLANTLETS

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Article Info: Received 23 January 2019; Accepted 16 February, 2019

Cite this article as: R, J., K M, S. M., ., H., & ., V. (2019). RAPID *IN VITRO* PROPAGATION OF WESTERN GHATS CULTIVAR - *COLOCASIA ESCULENTA* FOR THE HIGH FREQUENCY OF PLANTLETS. *Journal of Drug Discovery And Therapeutics*, 7(2).

DOI: <https://doi.org/10.32553/jddt.v7i2.447>

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Disclosure statement: The authors have no conflicts of interest.

ABSTRACT

Colocasia esculenta is commonly known as Taro, it is referred to as cocoyam in Nigeria. They are cherished for their rich taste, nutritional and medicinal properties. Every 100 g of taro corms possess 112 Kcal, 26.46 g carbohydrate, 1.50 g protein, 0.20 g total fat and 4.1g fiber (USDA National Nutrient Data Base). Besides its nutritional value, taro is used as a medical plant and provides bioactive compounds used as an anti-cancer drugs. Traditionally, cocoyams are vegetative propagated from tuber fragments, a practice that encourages pathogen distribution. *Colocasia esculenta* is a widely distributed food crop in the humid tropics and subtropics. Despite of its wide distribution, Taro plants are commonly infected with DsMV and other pathogens. This virus induces conspicuous mosaic, malformation, dwarfing or feathering on leaves in taro. As the results of infection, it reduces the quality and yield of taro production greatly. This virus is thus considered as a major limiting factor in the production of taro. Here plays the importance of tissue culture plays a major role in producing the disease resistant plants round the year with high quality. For rapid multiplication and production of quality planting materials, tissue culture technology offers promising alternative compared to the traditional production methods.

Keywords: *Colocasia esculenta*, Virus, Pathogens, Conventional propagation, Micropropagation, Yield, Rapid multiplication, Quality

INTRODUCTION

C. esculenta is commonly referred to as taro and it belongs to araceae family. The various parts are cherished for their rich taste and nutritional properties. Every 100 g of taro corms possess 112 kcal, 26.46 g carbohydrate, 1.50 g protein, 0.20 total fat and 4.1g fiber (USDA National Nutrient Data Base,1998). Besides its nutritional value, taro is used as a medical plant and provides bioactive compounds used for therapeutic purposes

(Prajapati *et al.*,2011). Traditionally, taro is vegetatively propagated from tuber fragments, a practice that encourages pathogen distribution. *C. esculenta* is a widely distributed food crop in the humid tropics and subtropics. Despite of its wide distribution, Taro plants are commonly infected by many pathogens. The virus induces conspicuous mosaic, malformation, dwarfing or feathering on leaves in taro. As the results of infection, it reduces the quality and yield of taro production greatly. This

virus is thus considered as a major limiting factor in the production of taro. Hence *in vitro* propagation plays a major role in producing the disease resistant plants round the year with high quality. The tissue culture technology offers promising alternative compared to the traditional production methods for rapid multiplication and production of quality planting materials. (Alfred *et al.*, 2015).

MATERIALS AND METHODS

Media preparation

Optimized Murashige and Skoog (MS) medium was used as the basal medium and was prepared according to the following composition:

Table 1 : Composition of nutrients of Murashige and Skoog (MS) medium

Nutrients	Conc. (g/L)
NH ₄ NO ₃	1.65
KNO ₃	1.9
MgSO ₄	0.373
KH ₂ PO ₄	0.173
CaCl ₂	0.44
MnSO ₄	0.022
ZnSO ₄	0.0086
H ₃ BO ₃	0.0064
CuSO ₄	0.0025
Ascorbic acid	0.04
Citric acid	0.02
Fe-EDTA	0.0367
Sugar	30

Table 2 : Composition of Vitamins in Murashige and Skoog (MS) medium

Vitamin Solutions	Volume (ml/L)
Potassium iodide	1
Thiamin HCl	1
Sodium molybdate	1
Glycine	1
Pyridoxine HCl	1
Nicotinic acid	1
Cobalt Chloride	1

The basal medium was supplemented with various plant growth regulators such as Kinetin, 2, 4-dichlorophenoxyacetic acid (2, 4-D), Naphthalene acetic acid in different concentrations to verify their callus induction properties. Initiation was commenced using the various media trials after the surface sterilization of explants and incubated in the growth room and their observance was noted every day in order to record the changes in their growth and to identify the contamination percentage.

Collection and sterilization of Explant

The fresh leaves from healthy plant of *Colocasia esculenta* were collected from the western ghats of Karnataka and the sterilization was performed and the survival of explants were observed for callus induction. The explants were then subjected to treatment with washing surfactant (Tween-20), which was added in drops and explants in the solution were agitated constantly for 10 minutes. Thereafter, the detergent was completely drained out from the explants by rinsing it with sterilized water to expel the microbial load and dust particles. Consequently, the explants were taken to laminar airflow cabinet for further sterilization.

After transmission to laminar airflow chamber, the explants were further sterilized with 0.1% mercuric chloride (Ritu *et al.*, 2013). The different trials with

series of three, five and seven minutes time of exposure to the 0.1% mercuric chloride was carried out. After this treatment, explants were thoroughly washed 2 times with sterilized water to evacuate any trace of the surface sterilants under aseptic conditions. The sterilized explants were inoculated on MS media and kept for incubation at 37°C up to 30-45 days with regular monitoring in every 2 -3 days.

Shoot proliferation

After incubation, the growth of shoots was monitored. The individual shoots, which formed at the end of establishment stage, were re-cultured on MS media having 0, 1.0, 2.0, 3.0, 4.0, 5.0 mg/l of benzylamino purine (BAP). Each bottle was containing 35-40 ml of medium. The pH was adjusted at 5.6-5.8, while they were autoclaved at 121°C under a pressure of 1.5 kg/cm² for 20 min. The duration of multiplication stage was four subcultures. Each subculture period

ranged from 30-45 days. Number of shoots at the end of each multiplication subculture was recorded.

ROOTING:

The proliferated shoots were induced in the rooting media with various growth regulators for the emergence of roots. After 4 weeks, the percentage of rooting, number of roots and root length were recorded. The well-developed, fully elongated roots and shoots were then acclimatized for Hardening.

CULTURE CONDITIONS:

The culture bottles were subjected to light intensity for 10-12 h in the growth room for rapid multiplication. Photoperiod provided by cool white fluorescent lamps of 1500-3000 lux, temperature of about 25 ± 2 °C and humidity of 35 - 40%.

RESULTS AND DISCUSSION:

STERILIZATION SURVIVAL:

The explants were found to survive at 7 min treatment of Mercuric chloride.

Table 1: Sterilization Survival

TREATMENT OF MERCURIC CHLORIDE (%)	NO.OF EXPLANTS TAKEN	MORTALITY RATE (%)	NO OF EXPLANTS NOT RESPONDED (NOS)	BUD BREAK (%)
0.1% HgCl ₂ – 3 min	20	86±2.3	12	2±1.2
0.1% HgCl ₂ – 5 min		51±3.3	45	4±0.3
0.1% HgCl ₂ – 7 min		20±3.5	18	62±3.2

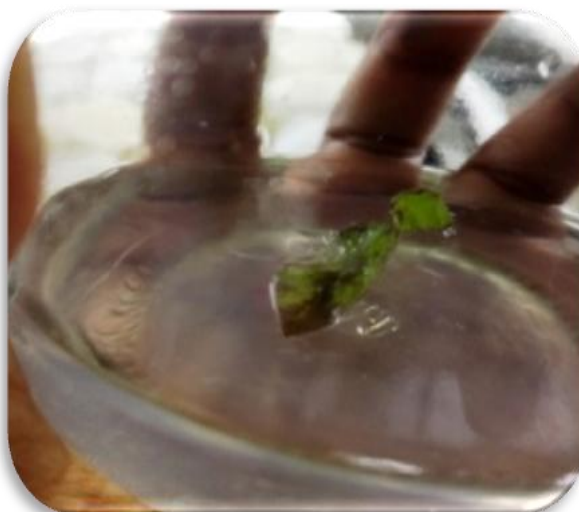


Figure 1: Survived Explants

Majority of the explants survived after treatment when they were exposed to mercuric chloride for about 7 min. Treatment with 3 min were found with more mortality of the explants whereas treatment at 5 min found to be non-responsive.

INITIATION MEDIA TRIALS:

Table 2: success rate of initiation

Hormone	Concentration	No. of explants responded	No. of days
Kinetin	1 mg/L	-	-
	2.5 mg/L	3	12 days
	3 mg/L	2	12 days
2,4-D	4 mg/L	3	6 ± 1 days
NAA	1 mg/L	3	10 days

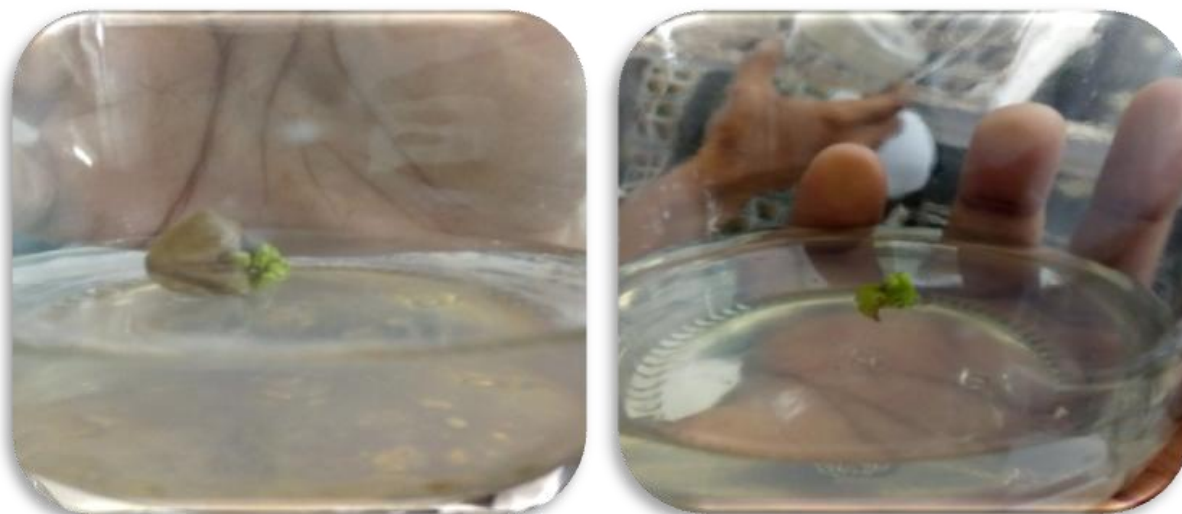


Figure 2: INITIATION RESPONSE - Kin – 2.5 mg/l

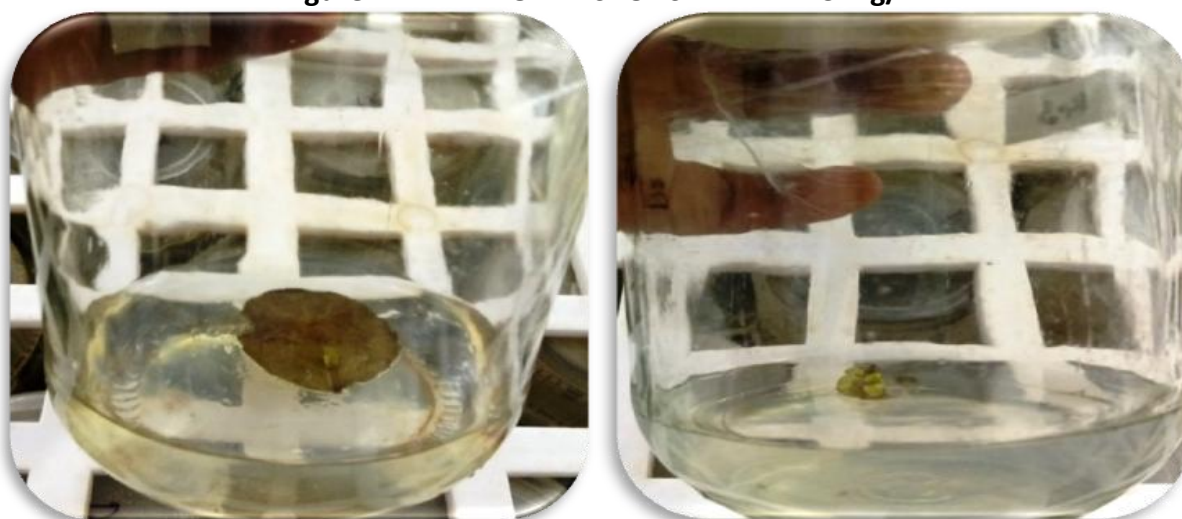


Figure 3: INITIATION RESPONSE - Kin – 3 mg/l



Figure 4: INITIATION RESPONSE - NAA – 1 mg/l

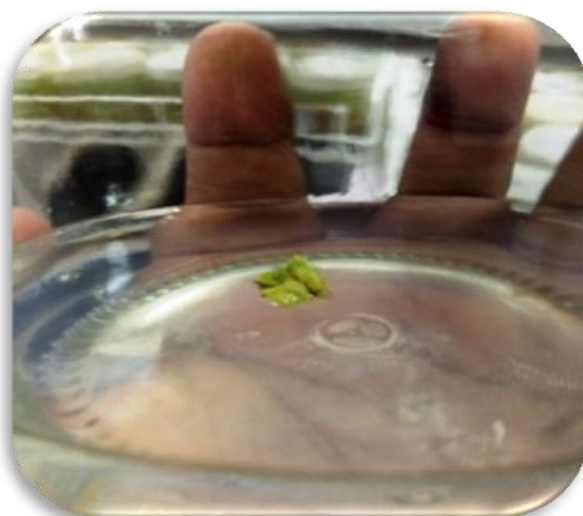


Figure 5: INITIATION RESPONSE - 2,4-D – 4 mg/l

PROLIFERATION RATE:

Using 6BAP, the responded explants were transferred to the proliferation media where the response was recorded at 5 mg/l 6BAP that led to 76% response followed by 1.58 multiplication ratio unlike the other concentrations. At lower concentration, there was least response with increase in the response rate as the hormone cytokinin concentration increased.

Table 3: Proliferation Response

Hormone	Concentration	% response	Multiplication ratio
6BAP	1 mg/L	21	0.8
	2 mg/L	30	0.91
	3 mg/L	42	1.06
	4 mg/L	59	1.58
	5 mg/L	76	2.2

**Figure 6: MULTIPLICATION RATE****Fig 7: MULTIPLICATION WITH CALLUS**

ROOTING:

Percentage of roots obtained was significantly different based on the concentrations of IAA and IBA and found about 78% efficiency with increased length of the roots of 3.2 cm.

Table 4: Root Induction Efficiency

Hormones	Concentration	Rooting efficiency (%)	Root length (Cm)
IAA	0.5 mg/L	34	1.1
	1 mg/L	30	0.41
IBA	0.5 mg/L	78	3.2
	1 mg/L	60	2.3

**Figure 8: ROOT INDUCTION****CONCLUSION:**

This protocol offers potential *in vitro* system that can be used for improvement, conservation and efficient mass multiplication of clones of *Colocasia esculenta* to prevent from an endangered and endemic species. Further, *In vitro* mass propagation reduces cost and completion of life cycle can be attained within reasonable time, reducing risk of extinction to the endangered wild population.

ACKNOWLEDGEMENT:

The authors record their sincere thanks to Chairman and Faculties of Department of Biotechnology and Microbiology. Many thanks to Shri. Gopala Krishna Bhat, Retd. Prof. Udipi, Mrs. Prasanna Bhat, Udipi, Dr. Rama Rao, Regional Ayurveda Research Institute for Metabolic disorders, Bengaluru for their timely support. A special thanks to Dr. V. Palani, Director

and Ms. Parvathy. S, R and D Manager , Genewin biotech for their support throughout the work.

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