African Journal of Biotechnology Vol. 7 (1), pp. 041-043, 4 January, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Short Communication

In vitro micropropagation of white dasheen (*Colocassia esculenta*)

Chien-Ying Ko[1]*, Ji-Ping Kung[1] and Rohan Mc Donald[2]

[1]The International Cooperation and Development Fund (Taiwan ICDF), Taipei 11157, Taiwan.
[2]Ministry of Agriculture, Forestry and Fisheries, St. Vincent and the Grenadines, Richmond Hill, Kingstown.

Accepted 14 November, 2007

Plant regeneration for efficient vegetative propagation in white dasheen has been developed. Meristem cultured on Murashige and Skoog medium supplemented with 6-Benzyladenine (BA) and Indole-3-acetic acid (IAA) gave the best explants establishment and development. This *in vitro* system provides for a uniform production system which is easily managed and yields high quality cornels for commercial production.

Key words: White dasheen, mass propagation.

INTRODUCTION

White dasheen (*Colocassia esculenta*) is one of the most important economic crops for starchy food in the eastern Caribbean (Archibald, 2002). Over the last ten years the government of Caribbean countries has embarked on an agricultural diversification program centered on bananas. Integral to this thrust is the renewed emphasis on root crops. Dasheen plants are considered the potential root crop in Caribbean countries such as St. Vincent, Dominica, St. Lucia with mass exports to the United Kingdom, France, Holland and Unite States of America (Archibald, 2003). In recent years, these countries have been increasing their demands and this has resulted in a five-fold increase in dasheen exports during the past 10 years (Robin, 2004).

Conventional method of white dasheen cultivation is being used via vegetative propagated plantlet for the field cultivation. The division of offshoots dasheen is not always suitable for this cultivation due to the weakness and susceptibility to pathological agents. White dasheen is one of the principal root crops that have shown great promise in generating income within the rural communities. However, the limited availability of proper planting material has imposed an emerging problem. To address this problem, *in vitro* micropropagtion was adopted with the objective of making dasheen planting material available to farmers. Micropropagation of dasheen plants has been reported through protocorm-like bodies (Sabapathy and Nair, 1992) and callus culture (Yam et al., 1990; Gupta, 1985) and subsequent regeneration of adventitious plantlets. Concern for somaclonal variation in the regeneration system through callus culture and protocorm-like bodies induce has been reported in other crops (Ahloowalia, 1987; Chatterjee and Gupta, 1997; Jaligot et al., 2000) and encountered based on our experience (Ko C-Y, unpublished). Therefore the development of a stable micropropagation procedure by shoot multiplication seems to be promising for business production system and the production quality in the future. Here, we report a simple, economical and rapidly multiplying protocol for mass propagation of white dasheen.

MATERIAL AND METHODS

Mature dasheen plants between 7 - 8 months old are used for initiation. Plants were washed with tap water and outer leaves are removed until inner cleaner section appeared. Plants were then surfaced-wiped with 70% ethyl alcohol. Outer leaves were separated from the dome in a circular fashion using a sterile surgical knife under a laminar flow. Meristem domes of about 1 cm^2 were surface sterilization in 0.5% sodium hypochlorite containing a few drops of Tween 20 for 8 min and rinsed 4 - 5 times with autoclaved double distilled water. The meristem explants were subsequently cultured on MS medium (Murashige and Skoog, 1962) supplemented with

*Corresponding author. E-mail: a0955579973@yahoo.com.tw. Tel/Fax: 966-553111068.

Table 1. Effect of BA and NAA combination on proliferation of white dasheen shoots.

Hormone (mg/l)		Shoot formation (%)	Ave Shoots/explant
BA	IAA		
2	0.5	30.5[c]	1.1[b]
4	1	70.0[b]	2.0[b]
6	2	90.0[a]	3.8[ab]
8	3	99.5[a]	5.9[a]

Mean separation in columns by Duncan's multiple rang test at P < 0.05.

5 mg/l benzylaminopurine (BA), 1 mg/l indole-3-acetic acid (IAA) and 30 g sucrose/l. The medium was solidified with 0.7% agar (Sigma Chemical Co., USA) and pH adjusted to 5.7 prior to autoclaving at 121°C for 20 min. All chemicals used were of analytical grade (Sigma Chemical Co., USA). The cultures were kept at 25 ± 2 °C, under 12 h (day) /12 h (night) photoperiod with light source provided by irradiation intensity (GE lighting, Daylight, F40D-EX) of 2000 - 2200 lux.

To screen for an optimal shoot multiplication medium (Table 1), single shoot explants of white dasheen were cultured on the MS basal medium supplemented with different concentrations of BA (2.0 - 8.0) and IAA (0.5 - 3.0). Ten single shoot explants were placed in one glass flasks containing 100 ml of the media. Each treatment was replicated four times. The culture condition was the same as described above. Shoot formation was recorded after 4 weeks. The data were analyzed statistically using a package by CoStat (Cohort Software, Minneapolis, MN).

Regenerated shoots were cultured onto MS medium supplemented with 0.25 mg/l α-naphthaleneacetic Acid (NAA), 1.5 g/l charcoal and 30 g/l sucrose for the induction of roots. The *in vitro* grown plantlets were then transferred to greenhouse under 70% shade and planted in 1.5 inches plastic trays with combinations of there difference potting media (Peat moss+Vermiculite+Perlite, 1:1:1 ; Peat moss + coconut fiber, 1:1 ; coconut fiber). Each treatment was 300 plantlets. Survival plantlets were recorded after 3 weeks [Survival plantlet (%) = (Survival plantlets / Total plantlets) x 100).

RESULTS AND DISCUSSION

Meristem bases cultured on 5 mg/l BA and 1 mg/l IAA supplemented MS medium turned green after culturing for one week. Multiple shoots were obtained after 2 - 3 weeks of culture on the same medium (Figure 1a). Subculture of these meristem domes were repeated every 4 weeks. These explants were used for multiplication shoots experiments.

Explants were cultured on MS media containing various combinations of BA and IAA to induce shoot proliferation of white dasheen. The results showed that shoot proliferation could be induced on most media. The frequency of shoots formation was between 30.5 - 99.5% and the best responded explants producing highest number of shoot (5.9) were in MS medium plus with BA (8 mg/l) and IAA (3 mg/l) as compared to other combinations (Table 1). Previous report indicated rapid vegetative multiplication of taro by adding TDZ, an artificial cytokinin with strong activity. However whether this regeneration procedure caused a somaclonal variation has not been mentioned

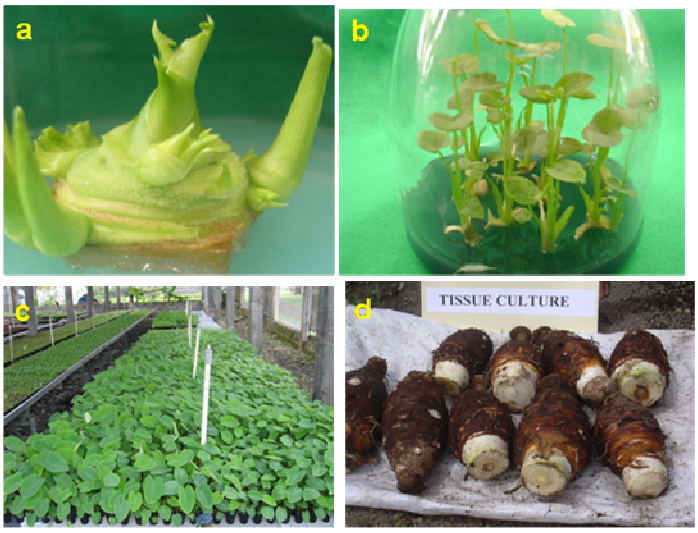

Figure 1. Production system of white dasheen by tissue culture. **a.** Meristem placed in medium which induces shoot. **b.** Medium used for establishment of proper rooting system. **c.** Hardening process and uniform production of white dasheen established from tissue culture plantlets. **d.** White dasheen corms harvested from tissue cultured dasheen plants.

(Chand and Pearson, 1998). Stieve et al. (1992) indicated tissue culture induced plants of *Zinnia marylandica* has more variation than seed-derived control plants depending on the concentration of TDZ. In commercial laboratories, a stable micropropagation process is required by controlling the subculture time, the part of explants, growth regulator concretion and category and the genetic background of plant varieties with all characteristics to maintain the quality of the product.

Subculture of the meristem domes were achieved after 3 weeks and until the sixth subculture. The seventh subculture was transferred onto a MS medium supplemented with 0.25 mg/l NAA, 1.5 g/l charcoal and 30 g/l sucrose for inducing adventitious roots (Figure 1b). Roots were initially induced on cutting surfaces of shoot base 5-7 days after culture. The Plantlets were maintained in the same medium and extra time of illumination so that they may develop proper rooting systems. The *in vitro* grown plantlets were then transferred to greenhouse under 70% shade and planted in 1.5 inches plastic trays (Figure 1c). Three media were used to compare survival percentage of plantlets during the hardening process. The results showed that plantlets could be survived on all test media, especially with peat moss, vermiculite and perlite mixture (1:1:1 ratio). The frequency of survival rate was between 86.2 - 96.1% (Table 2). Using coconut fiber alone can also maintain an above of survival 86%. This medium is popular for the growth of tissue culture plants such as banana and pineapple in our laboratory. Coconut medium is used to assist in the growing of plants and to provide them with the best physical growing environment. It is a very important recycle resource of agriculture material in

Table 2. Effect of media combination on the rate of survival plantlets.

Potting media	Media ratio	Survival plantlet (%)
Peat moss+Vermiculite+Perlite	1:1:1	96.1
Peat moss + coconut fiber	1:1	92.5
Coconut fiber	All	86.2

Survival plantlet (%) = (Survival plantlets /Total plantlets) x 100.

tropical and subtropical area.

We made use of this method to provide tissue culture plantlets to the Caribbean Agricultural Research and Development Institute (CARDI) for production comparison. The growth, chemical analysis and yield of dasheen planted with traditional planting material and tissue culture plantlets were compared. The result showed that dasheen grown from both source had a similar composition, growth and yield with no difference due to method of propagation (Archibald, 2003, 2005). This *in vitro* system provides for a uniform production system which is easily managed and yields high quality corms (Figure 1d). In this report, we have developed the feasibility of using white dasheen for induction of multiple shoots *in vitro*. This achievement can be applied to commercial mass production and provides a uniform product quality.

ACKNOWLEDGEMENTS

This work was supported by grant to Chien-Ying Ko from the Taiwan ICDF and Ministry of Agriculture, Forestry and Fisheries, St. Vincent and the Grenadines. We thank Chris Lee for reviewing of the manuscript.

REFERENCES

Archibald K (2002). Caribbean Agricultural Research and Development Institute (CARDI). Ann. Rep., pp. 10-11.
Archibald K (2003). Caribbean Agricultural Research and Development Institute (CARDI). Ann. Rep., pp. 10-12.
Archibald K (2005). Caribbean Agricultural Research and Development Institute (CARDI). Ann. Rep., pp. 6-11.
Ahloowalia BS (1987). Plant regeneration from embryo-callus culture in barley. Euphytica. 36: 659-665.
Chatterjee B, Gupta PD (1997). Induction of somaclonal variation by tissue culture and cytogenetic analysis in *Oryza sativa* L. Biol. Plant. 40: 25-32.
Chand H, Pearson MN (1998). Rapid vegetative multiplication in *Colocasis esculenta* (L) Schott (taro). Plant Cell Tissue Organ Cult. 55: 223-226.
Gupta PP (1985). Plant regeneration and variabilities from tissue cultures of cocoyams (*Xanthosoma Sagittifolium* and *X. Violaceum*). Plant Cell Rep., 4: 88-91.
Jaligot E, Rival A, Beule T, Dussert S, Verdeil JL (2000). Somaclonal variation in oil palm (*Elaeis guineensis* Jacq.): DNA methylation hypothesis. Plant Cell Rep., 19: 684-690.
Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15: 473-497.
Robin G (2004). Dasheen (*Colocasis esculenta* (L) Schott var. *esculenta*): research in Dominica to address constraints to production. A compilation of CARDI research paper. 4: 1-11.
Sabapathy S, Nair H (1992). *In Vitro* propagation of taro, with spermine, arginine and ornithine. Plant Cell Rep. 11: 290-294.
Stieve SM, Stimart DP, Yandell BS (1992). Heritable tissue culture induced variation in *Zinnia marylandica*. Euphytica. 64: 81-89.
Yam TW, Young JLP, Fan KPL, Arditti J (1990). Induction of callus from axillary buds of taro (*Colocasis esculenta* var. *esculenta*, *Araceae*) and subsequent plantlet regeneration. Plant Cell Rep., 9: 459-462.