"MERICLONING" OF TARO (*COLOCASIA ESCULENTA*)*

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SUMMARY

Calluses from mature taro tissues are difficult to obtain, but shoot production can be induced with the shoot meristem technique. To achieve maximum growth and multi-propagation of desirable clones, an effective procedure appears to be a combination of the shoot meristem technique and a supplementary treatment for callus formation.

RESUME

Il est difficile d'obtenir des cals des tissus du tarot, mais on peut provoquer la produccion des pousses par la méthode de méristéme appliquée aux pousses. Pour obtenir la croissance maximale et la pluripropogation de clónes désirables, il se révèle efficace de combiner la méthode de méristème des pousses à un traitement supplémentaire pour la formation des cals.

RESUMEN

Los callos de tejidos maduros de malanga son dificiles de obtener, pero la producción de follaje se puede inducir con la técnica de meristemos del follaje. Para lograr el máximo crecimiento y propagación de los clones deseables, la combinación de la técnica de meristemos del follaje y un tratamiento suplementario para la formación del callo, parece ser un procedimiento efectivo.

INTRODUCTION

Aseptic propagation of monocots has been achieved with members of diverse families. Among them are orchids[10,13,18] asparagus[8,14,15,17], sugarcane[1,5], grasses[7] (and unpublished data of U. Urata), oats[2], rice[12] and bromeliads[9]. More recently, Hartman and Zettler[3] reported that meristem-tip cultures of aroids could be successfully used for rapid propagation and for obtaining pathogen-free plants[4]. For rapid multiplication of desirable clones of taro it would be useful to find rapid means of propagation.

Attempts were made in Hawaii by Kikuta and Parris[6] to multiply planting material rapidly by propagating from the axillary buds on the mature corm and on 'huli' (a Hawaiian word for taro stem cuttings containing 1.2 mm of the corm tip and 15 to 25 cm of petiole base). Their idea was to stimulate the growth of normally dormant axillary buds. By destroying the growing point of the corm, the apical dominance inhibiting the development of the lateral buds was removed and a number of shoots developed. The present study began in 1972 assessing the feasibility of utilizing tissue cultures to obtain larger numbers of clonal plantlets of the taro cultivar 'Niue'.

MATERIALS AND METHODS

Taro cultivars were obtained from Lyon Arboretum of the University of Hawaii in 1971 and 1972. The following plants showing special features were selected for culturing.

Taro Niue, preferred Samoan taro,	Accession No L-69.500
Niue Uli, " " "	L-70.253
Eleele Naioea, somewhat resistant to *Phytophthora*,	No L-68.133
Makoko,	No L-69.047
Elapaio, an ornamental with variegated leaves.	No L-68.124
Uahiapele (Smoke of Pele), maroon coloured leaves,	No L-69.049
Manini Kea, an ornamental with white stripes on petiole,	No L-68.138

Preliminary studies with Taro Niue indicated that calluses were difficult to induce from various plant parts.

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Sections of petioles, corms, spadices (staminate and carpellary tissues) and roots were cultured aseptically on solid media in Falcon plastic, snap-on dishes. For surface sterilization, 10 to 15 percent chlorox solution was used. Cultural treatments were carried out in a Latin square design on Murashige and Skoog's basal medium[11], which was supplemented with gradients of coconut water (as a source of natural cytokinins) at 0, 2.5, 5.0 and 10 percent by volume, and auxins. Gradients for naphthaleneacetic acid (NAA) were 0, 1.25, 2.5 and 5.0 ppm, and for indoleacetic acid (IAA) were 0, 0.1, 0.5 and 1.0 ppm. These gradients were useful in studying auxin-cytokinin interactions with coconut water. The cultures were placed under continuous light and examined at intervals for callus formation. Additional tests were carried out in White's basal medium[16] supplemented at 10 percent by volume with coconut water, and with 2,4-dichlorophenoxyacetic acid (2,4-D) at 5 ppm, and adenine at 20 ppm. Unfortunately, none of the tissues responded positively to these treatments. Most of them turned dark brown. The spadix segments became large and swollen, probably due to the enlargement of the cells, but calluses were not formed.

Since the callus method on taro explants failed completely with the media tried, an alternative shoot meristem technique described by Sagawa *et al.*[13] was attempted. Shoot tips, rather than strictly apical meristems, of the taro cultivars listed above were cultured, both from the apical shoot and lateral buds. They were surface sterilized with 15, 10, 5 and 1 percent chlorox solutions, in descending order as the tender tissues become exposed when the outer leaves were carefully removed under the low power microscope. A drop of 'Ivory' liquid was added as a wetting agent into the 15 percent chlorox solution.

The cultures were grown in modified Knudson's C liquid medium supplemented with coconut water at 10 percent by volume, and by adenine at 20 ppm. About 10—15 ml. of the medium were pre-sterilized in 50 ml. Erlenmeyer flasks, and after inoculating one shoot tip per flask, the cultures were shaken continuously on the gyrorotary shaker under continuous light.

Difficulties were encountered in obtaining bacterial free cultures from shoot tips. The bacteria were probably lodged between the apparently tightly-folded young leaves. If endogenous, the same contaminant would have appeared in the plate cultures, but the plates were negative. When the liquid medium showed slight turbidity, the shoot tip was immediately removed and re-sterilized with 15 percent chlorox solution containing a drop of detergent. The material was next immersed in 5 percent and 1 percent chlorox solutions. In the latter one or two more leaves were removed aseptically, taking precautions not to injure the apical meristem proper. It was rinsed in sterile distilled water and transferred to a fresh medium. Tissue from a few of the contaminated flasks, after re-sterilization and trimming down of outer tissues, were stored overnight in a 20 ml. of sterile water containing a drop of 15 percent chlorox solution. The tissues remained alive while the contaminant was eliminated.

RESULTS AND DISCUSSION

After 6—8 weeks the cut ends of shoot tip explants showed visible proliferation of new tissues. When there was shoot elongation without proliferation at the base, the shoot tip was removed aseptically, washed in 1 percent chlorox solution and a fresh cut made closer to the apical meristem. After a quick rinse in sterile distilled water, the tip was inoculated into a fresh liquid medium and returned to the shaker. This extra step aided proliferation.

When the bases became swollen and protocorm-like bodies appeared, the cultures were transferred to solid media of different composition. Modified Murashige and Skoog's basal medium, supplemented with 10 or 20 percent coconut water by volume, supported excellent growth of the shoot tip as well as a ring of new plantlets at its base. Usually 4 to 8 plantlets appeared from the proliferated based upon transfer to a solid medium. Additional plantlets were formed in older cultures, particularly when tube cultures were transferred to flasks with fresh medium. Plate I shows three cultivars of taro with plantlets which were grown from shoot tip cultures: A, Taro Niue; B, Eleele; C, Uahiapele and D. Taro Niue. D is a shoot culture from one of 11 plantlets which proliferated from one shoot. A was grown with 10 percent coconut water while cultures B—D were grown with 20 percent coconut water in the basal medium. In A, the terminal shoot was accidentally decapitated at transfer, thus removing its apical dominance, and the lateral buds were stimulated to grow into shoots. Studies are in progress to find better media for inducing callus growth and unlimited production of plantlets after the shaker treatment, as was achieve with the pineapple[9].

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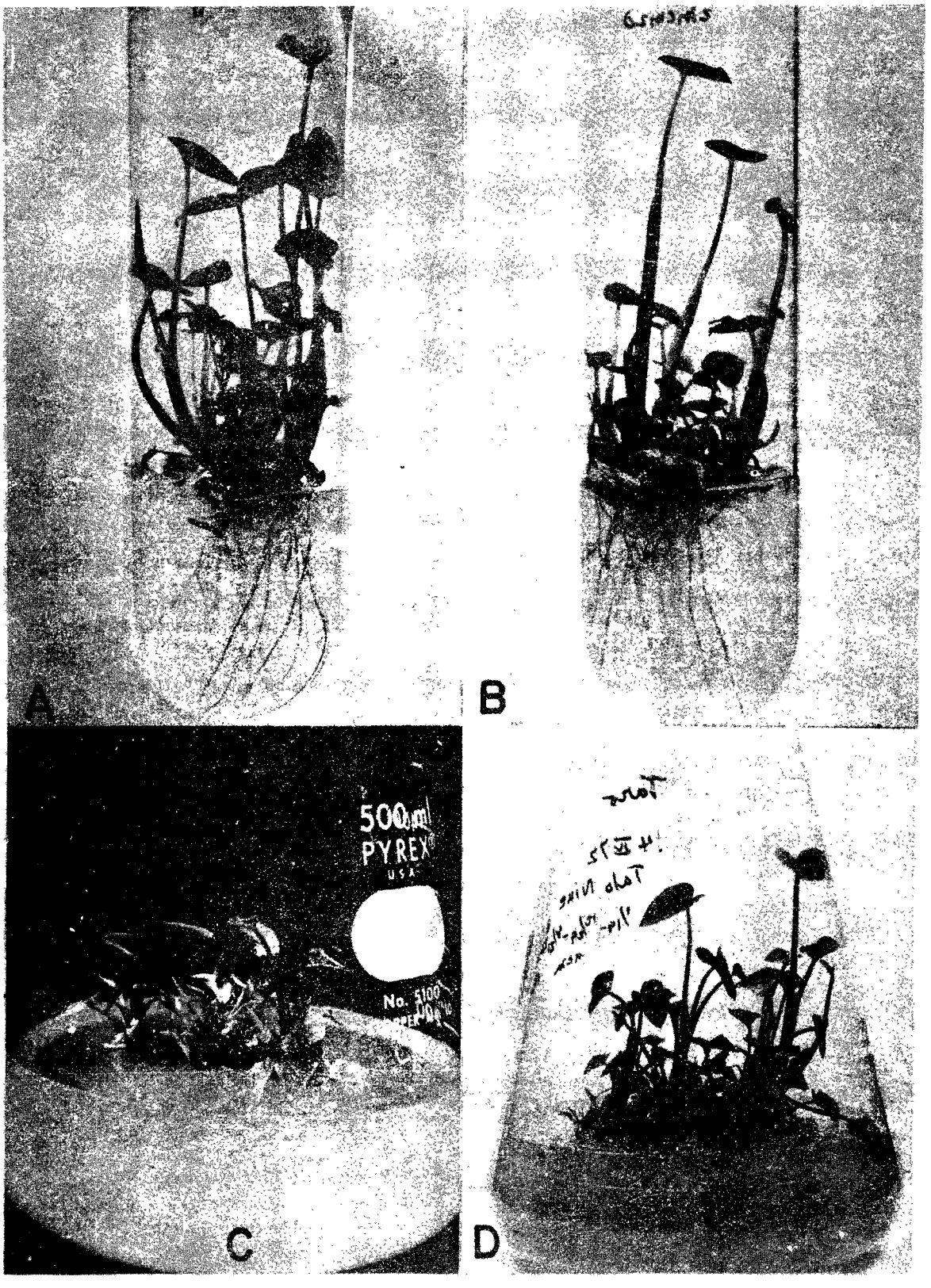

EXPLANATION OF PLATE *

Plate I. Growth of taro plantlets under aseptic conditions. Plantlets were obtained from shoot tip cultures after shaking them on the gyro-rotary shaker and transferring them to solid media. A. Taro Niue cultivar which produced several plantlets after the terminal shoot was decapitated at transfer, grown in Murashige and Skoog's (M–S) basal medium which was supplemented with coconut water, 10 percent by volume; B. Eleele cultivar with plantlets in M-S medium plus coconut water, 20 percent by volume; C. Uahiapele cultivar proliferating in a flask containing the same medium as in B; D. Taro Niue cultivar grown in the same medium as in B, grown from one of 11 plantlets which appeared in an earlier treatment with 20 percent coconut water.