Dasheen Mosaic Virus and Other Phytopathogens Eliminated from Caladium, Taro, and Cocoyam by Culture of Shoot Tips

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ABSTRACT

Excised shoot-tips of the aroids caladium, taro, and cocoyam, were successfully cultured aseptically and free of dasheen mosaic virus on the revised medium of Murashige and Skoog (14). Callus of all three species proliferated into numerous plantlets on this medium. Plantlets survived transplanting from culture to soil. Both caladium cultivars ('Candidum' and 'Frieda Hemple') assumed characteristic foliar variegation within 3 mo after transplanting. Candidum plantlets attained maturity and flowered 6-7 mo after transplanting. The feasibility of tissue culture as a practical control measure for diseases of these plants is discussed. Attempts to culture dieffenbachia, chinese evergreen, philodendron, and two *Cryptocoryne* spp. were not successful.

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Additional key words: Philodendron selloum, Araceae, Colocasieae.

Plants of the family Araceae are widely cultivated as ornamentals and for food. Among the important ornamentals are caladium (*Caladium hortulanum* Birdsey), *Dieffenbachia* spp., *Aglaonema* spp., and *Philodendron* spp. grown for their attractive foliage, and *Cryptocoryne* spp. used as aquarium plants. Other aroids, particularly *Colocasia* spp. and *Xanthosoma* spp. are important food staples of the tropics and subtropics (3, 11, 13, 15).

Most cultivated aroids are propagated exclusively by vegetative means. Although many can be grown from seed, special techniques must usually be employed to effect fertilization, and progeny do not necessarily reflect parental types (6, 12, 19).

Due to continued vegetative propagation of these plants, growers are experiencing increasingly serious disease losses. Dasheen mosaic virus (DMV), for example, appears to have become ubiquitous among cultivars of caladium, dieffenbachia, taro, and cocoyam (2, 5, 20). Similarly, it is impossible to accurately assess the pathogenicity of certain soil organisms to caladiums and other aroids due to the unavailability of pathogen-free material for testing (J. F. Knauss, H. N. Miller, and W. H. Ridings, *personal communications*).

Tissue culture techniques were considered as a practical means of eliminating pathogens from aroid planting stock. Tissues of numerous plant species have been cultured successfully in vitro, and consequently freed of pathogens (7). In some instances, tissue culture affords the sole means by which viruses can be eliminated from planting stock. This technique can provide the added advantage of being a useful means to mass-propagate slow-growing plants, such as orchids. This study was designed to obtain large numbers of pathogen-free aroids through shoot-tip culture.

MATERIALS AND METHODS.—Attempts were made to culture the following aroids in vitro: caladium (cultivars 'Candidum' and 'Frieda Hemple'), taro (*Colocasia esculenta* (L.) Schott), cocoyam (*Xanthosoma sagittifolium* (L.) Schott), dieffenbachia (*Dieffenbachia picta* Schott 'Exotica'), chinese evergreen (*Aglaonema modestum* Schott), philodendron (*Philodendron selloum* C. Koch), *Cryptocoryne cordata* Griff., and *C. nevillii* Trimen. According to Engler's classification system, caladium, taro, and cocoyam are representatives of the tribe Colocasieae; whereas dieffenbachia, chinese evergreen, philodendron, and *Cryptocoryne* spp. are in the tribes Dieffenbachieae, Aglaonemateae, Philodendreae, and Areae, respectively. All except *P. selloum* are propagated commercially exclusively by vegetative means, and all except *C. nevillii* have been shown to be susceptible to DMV (2, 4, 5, 9, 20, and R. D. Hartman, *unpublished*).

In this study, the "revised" medium of Murashige and Skoog (14) was used except that: *meso*-inositol (i-inositol dihydrate) was substituted for *myo*-inositol, Edamin was omitted, and each liter of medium contained 8 rather than 10 g of agar. The kinetin level employed was 1.0 mg/liter of medium and the indoleacetic acid level was 15.0 mg/liter.

Shoot-tips from all plants were excised and treated sequentially as follows: shoots were trimmed to $\leqslant 1.5\ cm^3$, rinsed in flowing tap water, submerged 10 min in 0.52% sodium hypochlorite, trimmed further, submerged for 5 min in 0.26% sodium hypochlorite, and rinsed briefly in sterile distilled water. With the aid of a dissecting microscope, each shoot was then trimmed until only the apical dome and one or two leaf primordia remained (Fig. 1-A, B). Finally, each shoot was transferred, cut surface downward, to the surface of 15 ml of solidified medium in a 30-ml, screw-cap vial. All cultures were maintained at 21-25 C and provided 12 h of daily light ca. 8,070 lx (750 ft-c) from incandescent and fluorescent bulbs.

Plantlets which had differentiated in culture, were

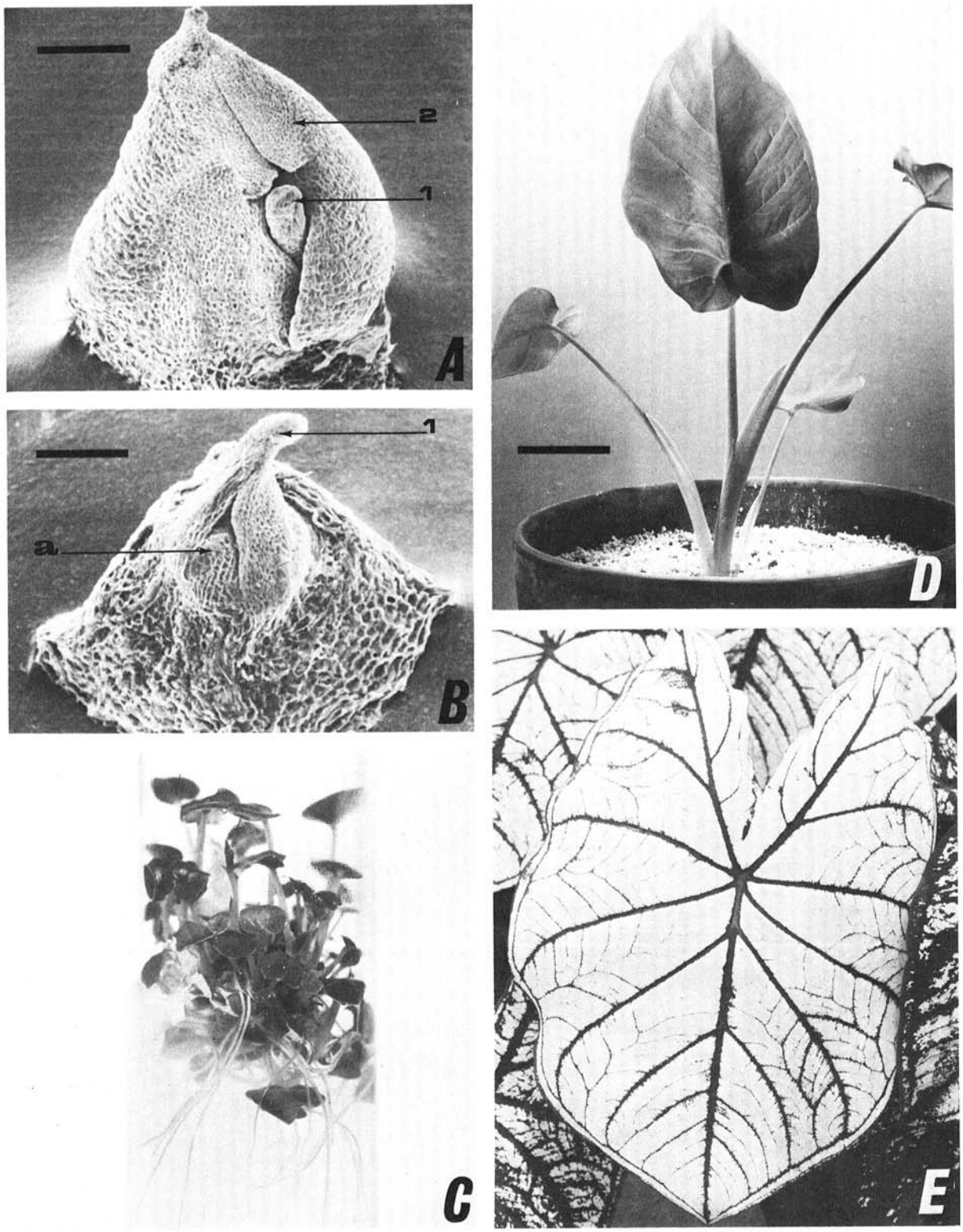

Fig. 1-(A to E). A, B) Scanning electron micrograph of caladium apical shoot showing first (1) and second (2) leaf primordia and apical dome (a). Scale line is 0.166 mm. **C)** Six-wk-old culture of 'Frieda Hemple' caladiums on Murashige-Skoog medium. **D)** Cocoyam plant 6 wk after transfer to soil from culture. Scale line is 5.4 cm. **E)** Mature pathogen-free 'Candidum' caladiums grown under greenhouse conditions.

separated from undifferentiated callus and transplanted individually to sterilized sand/peat (1:1, v/v) contained in 7.5-cm diam clay pots. All potted plantlets were placed in insect-proof cages in a growth room and observed for virus symptoms before being transplanted to larger pots and transferred to greenhouses.

Several authors (1, 2, 4, 8, 20) have observed intermittently expressed symptoms in DMV-infected plants; thus, infected plants can easily be overlooked. Special precautions were taken, therefore, to ascertain the presence or absence of infected material. Plants were maintained for at least 3 mo in observation cages before being transferred to greenhouses. Samples were further checked for DMV infection by electron microscopic examination of leaf dip preparations and/or by manual transmission attempts to seedlings of *P. selloum* (20).

RESULTS.—Attempts to culture pathogen-free plants of caladium, taro, and cocoyam were successful. Forty of 50, 3 of 6, 4 of 6, and 6 of 6 shoots of Candidum and Frieda Hemple caladium, taro, and cocoyam, respectively, developed in vitro without apparent microbial contamination. Shoot-tips, originally cream-colored, became green within 2 wk after excision. Within 8 wk, callus masses developed and differentiated numerous shoot buds. Within 18 wk, many plantlets with roots and shoots developed from these buds (Fig. 1-C). Plantlets of all three species survived transfer to soil and developed rapidly into vigorously growing plants (Fig. 1-D).

Every 3 mo, each caladium culture generated 10-20 plantlets suitable for transplanting, and enough callus for an additional 10-20 cultures. Candidum caladium callus has been maintained in culture for at least 1.5 yr without apparent adverse affects expressed by plantlets after transfer to soil.

All attempts to culture dieffenbachia, chinese evergreen, philodendron, and *Cryptocoryne* spp. were unsuccessful either because microbe-free shoot-tips could not be obtained, or because they failed to grow in vitro. Six of 60, 2 of 2, 2 of 3, 3 of 10, and 2 of 5 shoot-tips of dieffenbachia, chinese evergreen, philodendron, *C. cordata,* and *C. nevillii,* respectively, were without apparent microbial contamination. However, none of these developed callus or differentiated into plantlets. Shoot-tips, originally cream-colored, became green 1-2 wk after excision, but eventually became necrotic and died.

Attempts to culture dieffenbachia on other media were likewise unsuccessful. Three of 20 and 2 of 20 apical shoots proved to be free of microorganisms when placed on Vacin and Went (18) and Knudson C (10) medium, respectively. However, as with the Murashige-Skoog medium, none of the five shoots survived in culture.

Plantlets of Candidum caladium and taro obtained from some shoot-tips in culture proved to be infected with DMV. Virus-infected plantlets were readily detected in vitro and as plants growing in soil. These observations were confirmed through inoculations of *P. selloum* seedlings and electron microscopy. Plants of both caladium cultivars, taro, and cocoyam derived from other shoot-tips, however, proved to be free of virus and were used in continued propagations.

Virus-free plants of Candidum and Frieda Hemple caladiums developed typically variegated leaves 10-12 wk after being transplanted into soil. Some of the Candidum caladiums flowered 6-7 mo after transplanting and produced corms 4-5 cm in diam, with leaves 20 to 27 cm wide and 25 to 32 cm long (measured from lamella tip to point of petiole attachment) (Fig. 1-E). After 1 yr, these plants had corms ca. 9.5 cm in diam with a fresh harvest weight of ca. 200 g. Mature plants were identical to their commercial counterparts, except that they appeared to be more vigorous. In one experiment, 14 DMV-free and 14 DMV-infected plantlets were transplanted from culture to soil and maintained under identical growing conditions. After 6 mo, corm weights of healthy plants were significantly greater (t value = 2.6; $P = <5\%$) than those from diseased plants (56.9 and 40.2 g/corm, respectively).

Approximately 350 virus-free Candidum caladium plantlets have been grown to maturity since this study was initiated. These plants were propagated in a screened greenhouse on raised asbestos-cement benches previously sterilized with a 5% commercial formulation of sodium hypochlorite. The plants were routinely treated with either nicotine sulfate or malathion for insect control and twice with Truban (5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole) for control of pythiaceous fungi. During their development, none of the plants ever had symptoms of DMV. At harvest (ca. 6 mo after planting), none of the plants had discolored or necrotic roots or corms.

DISCUSSION.—This paper reports successful attempts to obtain pathogen-free plants of caladium, taro, and cocoyam through tissue culture techniques; attempts to culture dieffenbachia, chinese evergreen, philodendron, and *Cryptocoryne* spp., however, were unsuccessful. This appears to be the first report of the in vitro culture of aroids. Interestingly, the successes described herein involved plants of a single tribe, the Colocasieae; whereas, the failures involved representatives of other tribes in the Araceae. Success with these aroids may be contingent upon the use of other media. Even though shoot-tips of each of these aroids were isolated free of microorganisms, none survived and differentiated in vitro. Although the dissection of larger tissue pieces presumably would enhance the probabilities of survival in vitro, the relatively high rates of microbial contamination, particularly with dieffenbachia, is a deterrent to this approach. Moreover, the use of larger tissue pieces would also decrease, if not preclude, the chances of obtaining apical shoots free of DMV.

The only means of completely eliminating all known pathogens from these plants while retaining their respective genetic integrity appears to be through tissue culture; other control practices, including heat treatment, are not effective in eliminating DMV (1). Pathogen-free material obtained through tissue culture can be used effectively in pathogenicity trials with specific disease agents. Plantlets of Candidum caladiums were used, for example, in determining that *Pythium myriotylum* was a significant root pathogen whereas three other *Pythium* spp. commonly isolated from necrotic caladium roots were not (16).

Tissue culture may also be a valuable precautionary tool for those intending to introduce exotic species or cultivars of *Caladium, Colocasia,* or *Xanthosoma* into

new areas. Leon (11) called attention to the dangers of the inadvertent importation of pathogens in vegetative plant parts, and announced the need for defining areas where pathogen-free material could be obtained. Some pathogens, such as DMV, already appear to be distributed world-wide; indeed, this virus was detected in all accessions of cocoyam and taro at the Federal Experiment Station at Mayaguez, Puerto Rico (2). Other pathogens, however, such as the bacilliform viruses of taro described from the British Solomon Islands (4, 8, 9), may have a restricted range. Thus, culturing new accessions of these aroids in vitro, and screening plantlets before releasing them, would greatly minimize the possibility of accidental introduction of potentially dangerous pathogens into new areas.

This study also shows that in vitro propagation of caladium, taro, and cocoyam is much faster than conventional means. A newly developed caladium cultivar, for example, cannot be made available commercially until sufficient saleable stock can be produced which, according to grower estimates, usually takes 6-9 yr at which time enough material is available to plant 0.2024 ha (0.5 acres) with ca. 20,000 seed pieces. Considering that 10-20 plantlets can be generated in vitro every 3 mo, and that each plantlet can attain maturity 6 mo later, it would be theoretically possible to produce enough pathogen-free stock within 3 yr to supply the entire caladium industry of Florida consisting of an annual production of 40-50 million corms. A similar approach was considered for narcissus by Stone (17) who discussed the feasibility of replacing the entire stock of the Isles of Scilly with virus-free material.

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