EFFECT OF CONTAMINANTS IN TISSUE CULTURES OF TARO (*COLOCASIA ESCULENTA*)

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ABSTRACT

*Taro (*Colocasia esculenta *var.* esculenta *(L.) Schott) is easily cultured* in vitro. *Twenty five genetically different taro clones from the breeding program at Bubia Agriculture Research Centre, Lae, (Papua New Guinea) were cultured* in vitro *on modified Murashige and Skoog (MS) minimal organic medium. Endogenous contamination in taro tissue culture is not being properly acknowledged therefore this study was initiated to assess the type and rate of contamination. The rate of contamination was 56% of the total shoot tips cultured. When visually assessed, 16% of red, orange or yellow and 24% of clear or milky white coloured bacteria or yeast were observed which came out from the base of the explants. Fungal contamination on the media accounted for another 16% of the total cultures. It was shown that the presence of micro-organisms in cultures affected explants, eventually result in death when contaminated cultures were not transferred onto fresh medium. It was concluded that detection methods should be incorporated at all stages of tissue culture to avoid losses due to endogenous micro-organisms.*

Keywords: Colocasia esculenta, *endogenous contamination, tissue culture*

INTRODUCTION

The shoot tip of taro (*Colocasia esculenta* L. Schott.) can be easily cultured *in vitro*. The tissue culture method has enabled studies on plant physiology (Yam *et al.* 1990 a, 1990 b), metabolism, morphogenesis (Mostafa *et al. 1976*; Yam *et al.* 1990 a, 1990 b; Yam *et al.* 1991) and has enhanced elimination of plant pathogens (Jackson *et al.* 1977), preserve plant germplasm in limited space, help germplasm exchange and rapid micro propagation (Chng and Goh 1994) of plant tissues. Although several reports of taro tissue culture are available, information on plant losses during *in vitro* culture caused by microbial contaminants has hardly been given attention. Information of *in vitro* contaminants of food crop including taro in this country are yet to be identified and documented.

At Bubia it was found that micro organisms can live within plant tissues for longer periods *in vitro* without being pathogenic and show up in cultures during short environmental changes. The presence of micro-organisms in cultures may inhibit growth rate and decrease the potential of *in vitro* propagation. Contaminants could become pathogenic *in vivo* when the plants are introduced into another climate. Furthermore, metabolites of the contaminants could be toxic to the culture during short climate changes in the growth room. Leifert *et al.* (1991) mentioned that losses of plants from microbial contaminants outweighs other bacterial contamination observed in *in vitro* cultures. Deleterious pathogenic micro organisms which exist endogenously *in vivo* and are not detected and removed could become a treat during tissue cultures.

Because of the usually high death rate of taro explants in tissue cultures this investigation was initiated to record and describe casual endogenous microorganisms. The paper reports on contaminants observed on twenty five genetically different F1 taro hybrids in tissue cultures at the Bubia laboratory.

MATERIALS AND METHODS

First generation hybrid taro clones from the breeding program at Bubia Agricultural Research Centre served as explant sources. Meristem shoot tips of about 2 to 3 cm in diameter without the outmost whorl of petioles were washed with tap water. Hereafter they were

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submerged into a sterilent (Sodium hypochlorite; Household bleach; marvo - Linn brand) of 20% and 10% for 10 and 20 minutes respectively followed by 3 rinses of sterile distilled water before isolation. Shoot tips of 0.2 cm in diameter and 1.0 cm in height were isolated aseptically onto a growth medium after the excess tissues were removed at each decontamination stage. One explant of each hybrid was grown on Murashige and Skoog (MS) (1962) minimal organic medium containing 0.3 mg/l NAA and 1.0 mg/l BAP (E' Medium). They were kept under 16h days, a light intensity of 2.5 m W cm^2 (provided by 40 W GroLux fluorescent tubes) at median temperatures of 22 to 26°C. Though isolation and identification were not done, percentage contamination, description of contaminants, time of appearance after isolation and the site of contamination were noted. The contaminants were described as bacteria, yeast and fungi using the C.A.B edition of Plant Pathologists Pocketbook (1983) based on their morphological characteristics.

RESULTS

Observations of contamination made on the isolated taro clones *in vitro* is shown in Table 1. Out of the 25 taro shoots cultured 56% were contaminated by micro organisms. Of the affected shoots, 16 and 24% were

Table 1. **Cultured taro clones alongside observations of contamination.**

Taro clone Name	Time of detection after isolation (days)	Type of micro-organism (visual observance)	Colour of micro-organism	Site of contamination (explant/ media)	Time at death of explant (days)	Number affected (%)
AN1-1 AN21-1 AN22-1 AN24-1	4 - 5	fungus	initial white, then grey 4-5 days later	media	14-21 after outgrowth of fungus	16
AN2-1,AN3-1 AN9-1, AN11-1	10 -14	bacteria/ yeast	red/orange/ yellow	explant base under the media	14 - 21 after detection	16
AN10-1 AN12-1 AN13-1 AN17-1 AN23-1 AN25-1	2 - 3	bacteria/ yeast	clear initial then milky white	explant base under media	21-24 after detection	24
AN4-1,AN5-1 AN6-1,AN7-1 AN8-1 AN14-1 AN15-1 AN16-1 AN18-1 AN19-1 AN20-1	0	0	0	0	0	44

Note: Cultures that had no contamination are indicated with zero (0)

affected by red, orange, clear and milky white bacteria or yeast respectively. The other 16% contamination was due to a fungus on the media. The red, orange and yellow contaminant occurred as slimy exudate, firstly observed after 10 to 14 days of isolation at the base of the explant in the growth medium. Contaminated cultures changed growth medium colour from colourless to yellow 7 to 14 days after detection. The highest contamination were due to the bacteria or yeast that appeared as shiny colourless exudate at the base of the explant 2 to 3 days after culture initiation. The contaminants changed the growth medium colour to milky white after 21 days of detection. Fungal contamination first seen as white mycelium on the media, changed colour to grey/brown after 4 to 5 days and then eventually became black. The growth vile was covered with fungal mycelium within a week. All explants in contaminated cultures died when not transferred onto clean medium.

DISCUSSION

Most contamination problems arise when tissue culture methods used are inefficient in: sterilisation of explants, detection of micro-organisms in *in vitro* culture, aseptic handling of explant and the sterilisation of culture vessel, instruments and media. Contamination of 40% from the base of the taro shoot explants in this experiment suggest that several of the methods were not efficiently executed.

Improper handling and transferring of explant could be possible reasons for the fungus contamination. The frequent occurrence of the same bacteria or yeast like micro-organisms on several of the cultures suggests that these two types are common endogenous micro-organisms in taro at Bubia. The investigation cannot distinguish micro-organisms' specificity or preference to occur in particular clones. Since only one explant per clone was cultured, firm conclusions cannot be drawn.

Contaminants pathogenic to plants *in vivo* staying latent for longer periods when introduced into plant tissue cultures, must be considered a threat in the tissue culture of taro. Early detection of latent contamination is therefore essential to prevent losses due to such contaminants. Bacterial contaminants were isolated from established plant cultures which had been *in vitro* for longer than 12 months (Leifert *et al.* 1991). Bacteria or yeast contaminants observed in this experiment occurred within one month after isolation. The variation in emergence of contaminants in the cultures suggests that screening for contaminants or detection methods should be incorporated at all stages of tissue culture so that losses due to latent micro-organisms (esp. bacteria) can be avoided. The explants in the contaminated cultures eventually died, confirming deleterious effect of micro-organisms on explants in tissue cultures.

If investigations on endogenous micro-organisms in taro were to be carried out, emphasis should be put to identify, describe, and document these micro-organisms. Studies should also be done to find out the relationship of these micro-organisms to taro and their affinity for specificity or preference on the taro varieties.

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