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Biological contaminants associated with in vitro establishment of nursery-derived sweetpotato shoots

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Abstract

The production of virus-free sweetpotato planting material is essential for sustainable seed systems and commercial crop production. However, microbial contamination during in vitro establishment remains a major limitation to successful tissue culture propagation. This study aimed to identify the major biological contaminants associated with nursery-derived sweetpotato shoots during in vitro establishment and to determine whether the age of the shoot influences contamination levels.

Nodal shoots of the cultivar Minj Purple were collected from nursery-grown plants at 2, 6 and 12 weeks of regrowth. Explants were surface sterilised using a sodium hypochlorite-based protocol and cultured under standard in vitro conditions. Contaminated cultures were classified into broad microbial groups based on visible morphological characteristics.

Fungi, yeast and bacteria were identified as primary biological contaminants during culture establishment. The overall contamination frequency was 65.8%, with fungal contamination occurring most frequently (48.8%), followed by yeast (15.4%) and bacteria (1.6%). The age of shoots had no significant effect on contamination levels during in vitro establishment, whereas contaminant type significantly influenced contamination frequency, with fungi being the predominant contaminant.

These results provide baseline information on biological contaminants affecting sweetpotato establishment at Aiyura. Improved nursery sanitation, targeted fungal management and refined sterilization protocols are necessary to reduce contamination losses in resource-limited tissue culture systems.

Keywords: sweetpotato, in vitro establishment, biological contamination, fungi

Introduction

Papua New Guinea (PNG) is recognised as a secondary centre of sweetpotato diversity, and the crop is a primary staple for a large proportion of the rural population, especially in the Highlands region (FAO, 2009; Roullier et al., 2013). Sustained productivity of sweetpotato relies on the availability of virus-free planting material, free from viral pathogens that can accumulate during vegetative propagation (Chang et al., 2008; Clark et al., 2012).

Tissue culture plays a central role in the production of pathogen-free planting material by enabling rapid multiplication and facilitating downstream virus elimination procedures (Kyte & Kleyn, 1996; Dennien et al., 2013). However, biological contamination remains a major constraint during the initiation and establishment of in vitro cultures, often resulting in substantial loss of explants (Hammond et al., 2014; Rawal & Keharia, 2019). Contaminants may originate from the plant surface, internal tissues, nursery substrates, or the surrounding environment, and once established in culture, they are difficult to eliminate.

Various surface sterilization and control strategies have been reported for sweetpotato and other crops, including the use of sodium hypochlorite, alcohol pre-treatments, fungicides, and antibiotics (Amante et al., 2003; Masna & Sumaryono, 2020; Amissah et al., 2016). Despite these measures, contamination persists, particularly when explants are derived from field or nursery sources where microbial loads are high. Understanding the predominant types of contaminants present during in vitro establishment is therefore essential for developing effective and cost-efficient control strategies (Hammond et al., 2014; Sinta & Sumaryono, 2022).

At the Highlands Regional Centre Tissue Culture Facility in Aiyura, sweetpotato cultures are routinely established from nursery derived material. While contamination levels are recorded, the biological nature of contaminants associated

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with the age of shoots has not been systematically assessed. The lack of this information limits our ability to implement targeted management practices to reduce losses.

The objectives of this study were to (i) identify the major types of biological contaminants occurring during in vitro establishment of nursery-derived sweetpotato shoots and (ii) determine whether the age of nursery-derived shoots influences contamination levels during establishment. It was hypothesised that identifying the predominant biological contaminants associated with nursery-derived sweetpotato shoots would facilitate the development of improved contamination management strategies for producing virus-free planting material.

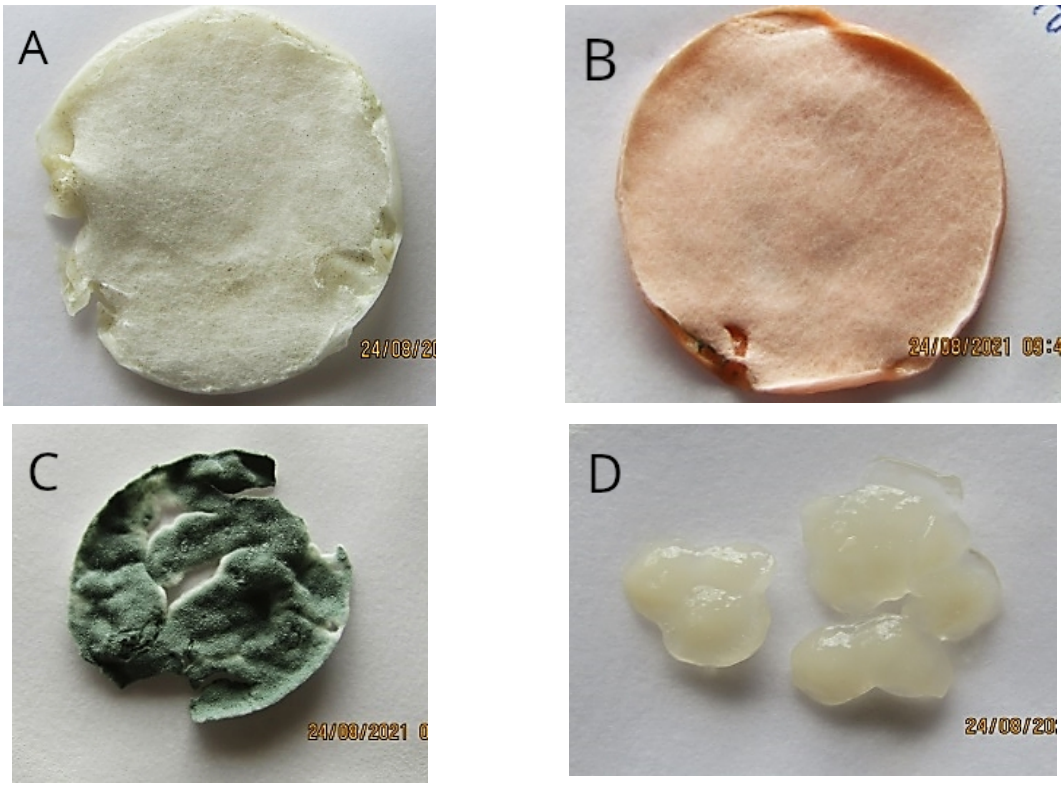

Figure 1. Representative biological contaminants recovered from contaminated *in vitro* sweetpotato cultures following sub-culture onto fresh sweetpotato medium (SPM). Photographs show the characteristic colony morphology observed after seven days of incubation at room temperature: (A) white filamentous fungal colony, (B) cream-coloured bacterial colony, (C) green sporulating fungal colony and (D) creamy bacterial colonies. Identification was based on colony morphology and growth characteristics. Scale bar = 1 cm.

Materials and Methods

Tissue culture establishment

Nine potted vines of Minj Purple sweetpotato (MPSP) were selected from nursery-established plants maintained since 2020. Vines were pruned at the base and allowed to regrow, and shoots were harvested at three age intervals: 2, 6, and 12 weeks.

Shoots containing 3–5 nodes were collected from each pot, placed in 500 mL culture containers, and transported to the laboratory for in vitro establishment. Surface sterilization was performed following the protocol of Dennien et al. (2013). Trimmed nodal explants were immersed and agitated in a soap solution for 15 minutes, followed by surface sterilization in 2% sodium hypochlorite solution for 15 minutes. Explants were then rinsed three times with sterile distilled water under a laminar airflow cabinet.

Under aseptic conditions, single nodal explants were excised and cultured individually in culture tubes containing sterile sweetpotato medium (SPM). A total of 108 cultures were established for each age group of the shoots (2, 6 and 12 weeks), giving an overall total of 324 cultures. Cultures were incubated at 22 °C under a 24 hour photoperiod.

Contamination Assessment and Identification of biological contaminants

Following weekly contamination assessments, the number of contaminated cultures from each age group of shoots was recorded. To confirm contaminant growth and facilitate

morphological identification, samples from contaminated cultures were aseptically transferred onto fresh sterile sweetpotato medium (SPM) in culture tubes and incubated for seven days at room temperature (Reina, 2016). Identification was limited to broad microbial groups because species-level identification was beyond the scope of this study and the laboratory facilities available.

Statistical analysis

Contamination count data were analysed using IBM SPSS Statistics version 20. Descriptive statistics were calculated to summarise contamination frequencies by the age of shoots and contaminant type. A two-way univariate analysis of variance (UNIANOVA) was performed to evaluate the effects of the age of shoots, contaminant type, and their interaction on contamination counts. Where significant differences were detected, pairwise comparisons of estimated marginal means were performed using the least significant difference (LSD) test at a significance level of $P \leq 0.05$.

Results

Table 1. Frequency (%) of biological contaminants observed during in vitro establishment of nursery-derived Minj Purple sweetpotato shoots.

Age of sweetpotato shoots	Fungal (%)	Bacterial (%)	Yeast (%)	Total contamination frequency (%)
2 Weeks	40.7	1.9	1.9	44.5
6 Weeks	49.1	2.8	37.0	88.9*
12 Weeks	56.5	0	7.4	63.9
Overall	48.8	1.6	15.4	65.8

*Percentages are not mutually exclusive because some cultures contained more than one contaminant type simultaneously. Therefore, contaminant frequencies should not be summed to estimate the proportion of contaminated cultures.

Table 2. Analysis of variance for the effects of the age of shoots and biological contaminant type on contamination counts during *in vitro* establishment of nursery-derived Minj Purple sweetpotato shoots.

Source	df	MS	F	p-value	Partial η²
Age (weeks)	2	64.33	1.72	0.207	0.161
Biological contaminant type	2	687	18.38*	<0.001	0.671
Age × Biological contaminant type	4	49.83	1.33	0.296	0.229
Error	18	37.37	—	—	—

Table 3. Least Significant Difference (LSD) pairwise comparisons of mean contamination counts among biological contaminant groups during in vitro establishment of nursery-derived Minj Purple sweetpotato shoots.

Biological contaminant Comparison	Mean diff. (I–J)	SE	95% CI	*P* < 0.05.
Bacteria vs.Fungi	-17.00	2.88	-23.05, -10.95	<0.001*
Bacteria vs. Yeast	-5	2.88	-11.05, 1.05	0.1
Fungi vs. Yeast	12.00	2.88	5.95, 18.05	0.001*

Note. Pairwise comparisons were performed using the Least Significant Difference (LSD) post hoc test following a significant main effect of biological contaminant type in the two-way univariate analysis of variance (UNIANOVA). Mean differences are based on contamination counts. Positive mean differences indicate higher contamination counts for the first contaminant group listed, whereas negative values indicate lower contamination counts. *Significant at P* < 0.05.

Three broad groups of biological contaminants; fungi, bacteria and yeast were identified from contaminated in vitro sweetpotato cultures following sub-culture onto fresh sweetpotato medium (Figure 1). Contaminated cultures exhibited either single or mixed microbial contamination. Single contamination consisted of fungi or bacteria, whereas mixed contamination comprised fungi with yeast or fungi with bacteria. Fungal contamination

occurred most frequently, either alone or in combination with yeast or bacteria.

Across the 324 cultures established, fungal contamination occurred most frequently (48.8%), followed by yeast (15.4%) and bacteria (1.6%). The overall frequency of contamination was 65.8% (Table 1). Contaminant frequencies were not mutually exclusive as some cultures contained more than one contaminant type.

There was no interaction between the age of shoots and biological contaminant type on contamination counts during in vitro establishment. Similarly, the age of shoots had no effect on contamination counts. In contrast, biological contaminant type significantly influenced contamination counts, indicating that the frequency of contamination differed among fungi, bacteria and yeast (Table 2).

Pairwise comparisons using the Least Significant Difference (LSD) test showed that fungal contamination counts were significantly higher than bacterial contamination counts (mean difference = 17.00, $P < 0.001$) and yeast contamination counts (mean difference = 12.00, $P = 0.001$). In contrast, bacterial and yeast contamination counts did not differ significantly ($P = 0.100$) (Table 3).

Discussion

Visual observations showed that fungal spores rapidly developed and multiplied on sterile media following culture initiation (Figure 1). These observations suggest that the surface sterilization protocol was insufficient to eliminate all fungal propagules present on nursery-derived shoots. Hammond et al. (2014) similarly reported fungal contamination as the predominant biological contaminant during in vitro establishment of sweetpotato cultures. They further noted that treatment with 0.2% mercuric chloride for 20 minutes reduced contamination levels during surface sterilization.

These findings indicate that improved contamination management strategies are required to reduce losses during sweetpotato in vitro establishment. Nursery management practices involving fungicide application prior to explant collection may reduce fungal contamination. Amante et al. (2003) discussed the use of thiabendazole for reducing fungal spores before planting vine cuttings, and this approach may be suitable for managing nursery plants prior to tissue culture establishment. Masna and Sumaryono (2020) also found alcohol pre-treatment to be effective when used before bleach sterilization and this may be incorporated into future sterilization protocols.

Strict nursery sanitation and aseptic handling procedures should also be maintained during field collection and transfer of explants into tissue culture facilities.

The use of antibiotics and fungicides during culture initiation may further minimise biological contamination. Amissah et al. (2016) reported that 0.5 gL^{-1} chloramphenicol and 0.1 gL^{-1} benomyl were effective against biological contaminants during sweetpotato tissue culture establishment. As prolonged exposure reduced culture growth rates, these compounds should only be applied during the initiation stage and omitted once clean cultures are established.

For bacterial contamination control, Izarra et al. (2020) transferred sweetpotato plantlets to soil and treated them weekly with Dimanin® at 2 mLL^{-1} before reintroducing shoots into in vitro culture. They reported complete elimination of latent bacterial contamination following treatment.

This study provides baseline information on the major biological contaminants affecting sweetpotato tissue culture establishment at Aiyura. The predominance of fungal contamination emphasises the importance of improved nursery sanitation, sterilization refinement and targeted fungal management strategies to improve successful establishment of clean sweetpotato cultures. Future work will investigate the use of additional antibiotics and fungicides and soil sterilization along with improved sanitation to improve the production of virus-free sweetpotato planting materials for the industry.

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Data availability. The data used to generate these results are available and can be accessed by liaising with the corresponding author.

Conflicts of interest. The authors declare that they have no conflicts of interest.

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