2024 SICASH
SLIIT INTERNATIONAL CONFERENCE ON ADVANCEMENTS IN SCIENCES & HUMANITIES

The Use of Plant Tissue Culture Techniques for Producing Virus Free *Manihot esculenta* Var. MU51 Plants

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Abstract

Even though *Manihot esculenta* (Cassava) has been identified worldwide as a key starchy crop, it contains a rich number of phytochemicals that can be used for various purposes. However, conventional propagation methods have led to an increase in viral diseases, creating a problematic condition when using this species for the pharmaceutical industry. There is a lack of reliable protocols for the micropropagation of cassava (Var. MU51) for mass production in industrial use. Therefore, this work aimed to develop a reliable method for the mass production of healthy, virus-free *Manihot esculenta* (Var. MU51) for industrial applications. The focus was on creating a micropropagation protocol for the variety MU51, involving optimal surface sterilization and effective hormonal combination for shoot proliferation and ideal media for meristem culture. According to the study, a 10% Clorox solution, coupled with an exposure duration of 15 minutes, manifested the most noteworthy success rate (78 %) in preventing contamination ($P < 0.05$). In the context of the proliferation of shoots from *M. esculenta* nodes, the application of 0.5 mg/l BAP + 0.1 mg/l NAA as well as 1mg/l BAP + 0.1mg/l NAA following 5 weeks, the nodal segments that underwent shoot proliferation exhibited comparable growth in both treatments without significant difference ($P > 0.05$). The initiation of meristem growth was carried out utilizing a solid Murashige and Skoog (MS) medium fortified with a blend of 0.1 mg/l BAP, 0.25 mg/l GA3, and 0.2 mg/l NAA, in addition to a standard MS medium. The hormonal MS medium demonstrated a significantly superior survival rate (87%) ($P < 0.05$). The current investigation underscores the optimal conditions for mitigating contamination risks and promoting desirable outcomes in *M. esculenta* shoot proliferation and meristem growth, thereby contributing valuable insights to the field.

Keywords: Cassava; In-vitro culture; Meristem culture; Micropropagation

1. Introduction

M. esculenta, scientifically classified as a perennial woody shrub typically cultivated annually, is a Manihot group member within the Euphorbiaceae family (Olsen and Schaal, 1999). With its starchy, swollen roots, *M. esculenta* is a staple food for millions in tropical regions and ranks as the third most important source of calories in the tropics, after rice and corn (Zeigler *et al*., 1980). This study focuses on *M. esculenta* variety MU51, which is valued for its high yield (35-40 t/ha), disease resistance, and low hydrogen cyanide (HCN) content of 40-45 mg/

https://doi.org/10.54389/WOFY1411

kg. *M. esculenta* contains phytochemicals such as flavonoids, tannins, anthraquinones, phlobatannins, saponins, and anthocyanosides, which provide diverse pharmacological benefits, including antioxidant, anti-inflammatory, antimicrobial, and anticancer functions (Scaria *et al*.,2023). However, *M. esculenta propagation* process presents a significant challenge in its production. Traditional propagation through stem cuttings often leads to viral and bacterial diseases, reducing crop productivity and risking the loss of superior genotypes (Nassar and Ortiz, 2007). Micropropagation addresses these issues by producing disease-free, genetically uniform plants, enhancing crop yield and quality. This method also has significant implications for the pharmaceutical industry, as it ensures the consistent production of cassava-derived pharmaceuticals. This highlights the need for alternative methods like micropropagation, a plant tissue culture technique. Micropropagation involves sterile cultivation and multiplication of cells, tissues, and organs using nutrient solutions, leveraging the totipotent nature of cells to regenerate entire plants from a single cell. Meristem culture, a type of micropropagation, is crucial for eradicating viruses from infected plants (Brar *et al*., 2021) and enhancing crop health and productivity. It involves extracting a small stem apex, eliminating pathogens, and preserving genetic stability (Hu *et al*., 1984). Exploring the benefits and processes of micropropagation will contribute to developing more efficient and disease-free methods of *M. esculenta* propagation and for phytochemical exaction.

2. Materials and Methods

This study was conducted at the plant tissue culture laboratory of the Department of Crop Science, Faculty of Agriculture, University of Ruhuna. Nodal segments of M. esculenta (Variety MU51) were used as the ex-plant materials. Surface sterilization procedures and medium preparation were carried out.

The culture procedure was conducted in a laminar airflow cabinet under aseptic conditions. During the surface sterilization process, various concentrations of Clorox (5%, 10%, and 15%) with TWEEN® 20 (0.1 mL) were applied for different exposure durations (10 minutes and 15 minutes). The non-contamination percentage was recorded after three weeks. In the second experiment, cultured nodal segments were transferred to a hormone-treated MS medium with combinations of BAP and NAA (T1: BAP 0.5 mg/l +NAA 0.1 mg/l; T2: BAP 1 mg/l + NAA 0.1 mg/l). The rate of shoot proliferation, survival percentage, and number of ex-plants were observed at weekly intervals. In the third experiment, the meristems were separated from the cultured ex-plants using a binocular stereoscopic microscope and transferred to a standard MS medium (T1) and MS medium fortified with plant growth regulators (T2: NAA 0.2 mg/l + BAP 0.1 mg/l + GA: 0.25 mg/l). The survival percentage of the meristems was observed in each treatment during 5 replicates. All experiments were carried out using a completely randomized design, and data were analyzed using the SPSS computer package following the ANOVA procedure. Mean separation was done using Duncan's Multiple.

3. Results and Discussion

3.1 Selection of Best Surface Sterilization Procedure for Ex-Plants

This study evaluated three distinct Clorox concentrations, each subjected to two different exposure durations following a 21-day incubation phase. The most effective sterilization method for M. esculenta nodal cuttings was the 15-minute exposure to 10% Clorox ($P<0.05$). However, 15% Clorox bleached some ex-plants, resulting in a lower non-contamination rate compared to the 10% Clorox treatment ($P<0.05$). This indicates that there is an optimal Clorox concentration for effective sterilization; surpassing this concentration could potentially be detrimental to the sterilization success process.

According to previous findings (Seessou *et al*., 2020), the effectiveness of a 20% Clorox solution for a 15- minute duration is considered the optimal approach for surface sterilization. The results of our study suggest that using a 15% Clorox solution for just 10 minutes is a similarly efficient combination for this application.

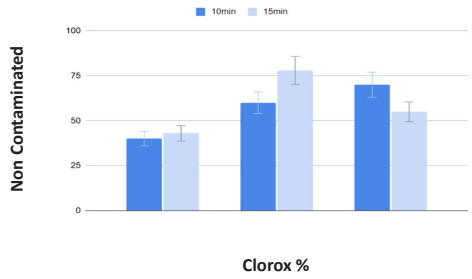

Figure 1. Effect of different sterilization methods on the non-contamination

3.2 Selection of best hormonal combination for shoot proliferation

In this study, two different concentrations of BAP were examined while maintaining a consistent level of NAA. The presence of NAA, along with BAP, promotes shoot proliferation (Ying *et al*., 2011). After three weeks post-insertion, it was observed that the ex-plants treated with both T1 (BAP 0.5 mg/l + NAA 0.1 mg/l) and T2 (BAP 1 mg/l + NAA 0.1 mg/l) demonstrated a similar proliferation rate ($P > 0.05$). The results suggest that both hormonal treatments, T1 and T2, were equally effective in promoting shoot multiplication over the studied period

Figure 2. Cassava noodle segments in different hormone media. A) T1 (BAP 0.5 mg/l + NAA 0.1 mg/l. B) T2 (BAP 1 mg/l + NAA 0.1 mg/l)

3.3 Survival percentage of meristem

The isolation and cultivation of meristems prove beneficial for obtaining plants free from pathogens. Following after standard tissue culture methods, meristems were grown on standard MS medium and MS medium enriched with BAP, GA3, and NAA.

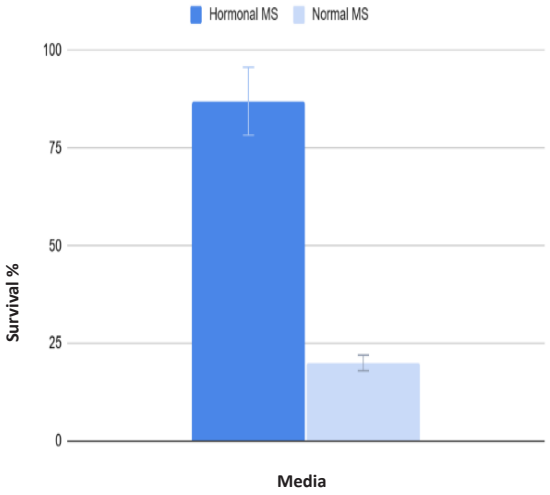

Figure 3. Survival percentage of meristems in different Media

The experiment showed that the standard MS medium led to a lower survival rate, while the hormonal MS medium significantly improved survival ($P < 0.05$). This suggests that for the *M. esculenta* MU51 variety, growth was faster and more beneficial in MS medium with BAP, GA3, and NAA, indicating these growth regulators are crucial for promoting and accelerating *M. esculenta* meristem growth.

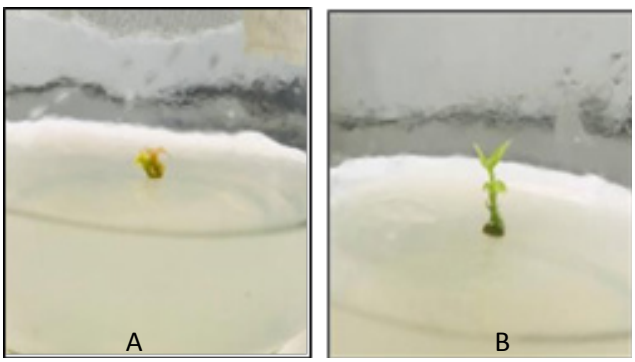

Figure 4. Meristems in different media A): Meristem in Normal MS. B): Meristem in Hormonal MS

4. Conclusion

The experiment established that a 10% Clorox solution for 15 minutes was the most effective sterilization method for *M. esculenta* nodal cuttings, showing a statistically significant improvement in sterilization efficacy ($P < 0.05$). Both hormone treatments, T1 (BAP 0.5 mg/l, NAA 0.1 mg/l) and T2 (BAP 1 mg/l, NAA 0.1 mg/l), produced similar proliferation rates after 3 weeks ($P > 0.05$). Additionally, the Hormonal MS media significantly improved the survival rate of *M. esculenta* plantlets compared to the standard MS media ($P < 0.05$). These findings highlight the importance of optimized sterilization and media composition for effective meristem culture and plantlet multiplication.

Acknowledgment

This research was funded by Starch Fruits & Beverages (Pvt. Ltd), Sri Lanka.

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