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OPTIMIZATION OF SURFACE STERILIZATION FOR AFRICAN VIOLET (*Saintpaulia ionantha*) IN TISSUE CULTURE

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ABSTRACT

Plant tissue culture studies, conducted under aseptic conditions, are of great importance for the rapid propagation of virus-free healthy plants and for various biotechnological applications. The success of such studies largely depends on the initial step of the culture process: surface sterilization of the explants. This study focused on the sterilization of initial plant material used in tissue culture applications of *Saintpaulia ionantha* H. Wendl. (African violet). Leaf tissue was used as the explant material. A total of five different sterilization protocols were tested. As a preliminary sterilization step, leaf explants were rinsed under running tap water for 30 minutes. For surface sterilization, explants were treated with sodium hypochlorite (NaClO) solutions at different concentrations (10–30%) and exposure times (10, 15, and 20 minutes). The effectiveness of various combinations using antibacterial liquid soap and Tween 20 was also evaluated. Following sterilization, the leaf explants were cultured on Murashige and Skoog (1962) nutrient medium without plant growth regulators. Initial signs of contamination were observed on the 6th day of culture. The most effective sterilization treatment was obtained with a solution containing 15% sodium hypochlorite and Tween 20, where 96% of the explants remained sterile and viable. These findings demonstrate the effectiveness of this protocol and provide a useful reference for the in vitro propagation of *Saintpaulia ionantha*.

Key Words: *African Violet, Ornamental plants, in vitro.*

INTRODUCTION

Saintpaulia is a genus within the *Gesneriaceae* family, comprising predominantly tropical herbaceous species and shrubs (da Silva et al., 2017). In tissue and cell culture studies, plant tissues and organs are propagated under aseptic and strictly controlled environmental conditions. The culture medium provides all essential nutrients required for plant growth and development. Such media typically comprise macronutrients, micronutrients, vitamins, plant growth regulators, a carbon source, and, in the case of solid media, gelling agents. Among these, the Murashige and

Skoog (MS) medium is one of the most widely used formulations for the in vitro vegetative propagation of numerous plant species. Depending on the application, both liquid and solid culture systems can be employed (Hussain et al., 2012; Bridgen et al., 2018).

In tissue culture, the presence of microorganisms is considered contamination, which represents one of the primary causes of culture losses. Contaminants may include viruses, bacteria, yeasts, and fungi (Oyebanji et al., 2009). Insufficient sterilization procedures can allow these microorganisms to proliferate. Sterilizing explants is particularly challenging when dealing with plant parts exposed to soil, species cultivated in tropical environments, or aquatic plants (Leifert, 2009). Additionally, certain species such as African violet, which possess a densely pubescent leaf surface, are inherently more difficult to sterilize. Furthermore, the nutrient-rich composition of plant tissue culture media—including minerals, vitamins, plant growth supplements, and sugars—can also facilitate the rapid multiplication of bacteria and fungi if contamination occurs.

Contamination by bacteria and fungi represents a persistent and frequent challenge in plant tissue culture systems (Misra and Misra, 2012). Once these microorganisms invade plant tissues or cell cultures, they rapidly proliferate, depleting nutrients from the culture medium. Their activity not only suppresses the growth and development of cultured plant tissues but may also lead to the release of toxic compounds that can ultimately kill the explants. Therefore, maintaining strict aseptic conditions and employing sterile techniques throughout all stages of in vitro plant culture manipulation is essential (Uğur et al., 2019).

This study aimed to optimize surface sterilization protocols for African violet (*Saintpaulia ionantha*), a species with a pubescent leaf surface that poses a high risk of contamination during in vitro propagation.

MATERIALS AND METHODS

This study was conducted on African violet (*Saintpaulia ionantha* H. Wendl.), a commercially cultivated ornamental plant. To optimize surface sterilization for in vitro propagation, five sterilization protocols were evaluated (Table 1).

Leaves were excised and initially rinsed under running tap water for 30 minutes. Subsequent sterilization steps were performed aseptically in a laminar flow hood. For all protocols, leaf explants were immersed in 70% ethanol for 1 minute, followed by three rinses with sterile distilled water. Treatment with 15% sodium hypochlorite (NaClO) solution was applied for varying durations, with or without pre-washing with antibacterial soap and addition of Tween 20, as summarized in Table 1.

After sterilization, leaf explants were cut into 1 cm^2 segments to expose leaf veins and used as explants for culture. The culture medium consisted of Murashige and Skoog (MS) basal salts supplemented with 3% sucrose (Duchefa), solidified with 0.7% agar (Duchefa), and supplemented with 2 mg/L 6-benzylaminopurine (BA), 0.5 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), and 0.03% activated charcoal. The medium pH was adjusted to 5.7 ± 0.1 before sterilization by autoclaving at 120 °C for 20 minutes.

Cultures were maintained at 24 ± 1 °C under a 16-hour photoperiod provided by white light-emitting diodes (LEDs). For each sterilization protocol, five replicates were prepared with four leaf explants per Petri dish to assess sterilization efficacy and explant viability (Figure 1).

Table 1**.** Surface sterilization protocols evaluated for African violet (*S. ionantha*) leaf explants.

Protocol	Antibacterial Soap	70% Ethanol	15% NaClO Treatment	Tween 20 Addition
1	–	1 min	10 min	–
2	–	1 min	15 min	–
3	–	1 min	20 min	–
4	+ (1 min)	1 min	10 min	–
5	+ (1 min)	1 min	10 min	+ (3 drops)

Figure 1. Visual representation of the sequential steps involved in the surface sterilization protocols applied to African violet (*Saintpaulia ionantha*) leaf explants.

RESULTS AND DISCUSSIONS

Bacterial and fungal contaminations pose significant threats in plant tissue culture systems. Plants can harbor bacteria and fungi from external sources, making the elimination of such contaminants one of the earliest and most critical steps in tissue culture protocols. In this study, contamination in leaf explants typically became visible around the sixth day after culture initiation. Bacterial contamination was more prevalent in the culture media, as illustrated. Although fungal contamination was less frequent initially, it became more apparent after the ninth day and extensively colonized the entire medium surface by the third week. The effectiveness of different surface sterilization treatments combining ethanol, varying durations of 15% sodium hypochlorite (NaClO), antibacterial liquid soap, and Tween 20 was compared to optimize decontamination for African violet explants. According to these results, Protocol 5, which includes antibacterial soap, ethanol, sodium hypochlorite, and Tween 20, achieved the highest percentage of sterile and viable explants (95%) and the lowest contamination rate (5%). This highlights the importance of combining multiple sterilizing agents and surfactants to enhance disinfection efficacy while maintaining explant viability (Table 2).

Surface sterilization remains a critical yet underexplored step in the in vitro culture of African violet (*Saintpaulia ionantha*). Despite the widespread use of this species for micropropagation and biotechnological applications, published protocols specifically addressing explant decontamination are scarce. However, some references have recommended the use of solutions such as tap water, 70% ethanol (v/v), autoclaved distilled water, commercial bleach solutions (e.g., “CLOROX®” containing 5.5% NaClO, diluted 1:5 with autoclaved distilled water), and Tween 20 (Fisher BioReagents, USA) for effective sterilization (Shukla et al., 2012).

Table 2. Contamination rates and explant viability following different sterilization protocols for African violet leaf explants.

Protocol	Contamination Rate (%)	Sterile but Dead Explants (%)	Sterile and Viable Explants (%)
1	90	10	-
2	60	20	20
3	-	100	-
4	75	25	-
5	5	-	95

Broadly, plant micropropagation protocols often utilize sodium hypochlorite within a 0.25–2.6% (w/v) range for 5–20 minutes, combined with a surfactant such as Tween-20 (1 drop per 100 ml), to mitigate surface contamination (Birmeta et al., 2022)

The inclusion of a surfactant (Tween 20), coupled with thorough pre-washing and sequential use of ethanol and sodium hypochlorite, significantly improved decontamination efficiency in our protocols. Protocols incorporating antibacterial soap pre-treatment, ethanol immersion, NaClO exposure, and Tween 20 achieved up to 95% sterilized and viable explants with minimal contamination. This synergistic strategy aligns with general best practices for micropropagation and highlights the need for surfactant inclusion in species with pubescent or hydrophobic leaf surfaces.

CONCLUSIONS

This study highlights the critical importance of optimizing surface sterilization protocols for successful in vitro propagation of African violet (*Saintpaulia ionantha*). Among the tested protocols, the combination of antibacterial soap, ethanol, sodium hypochlorite, and Tween 20 provided the most effective decontamination, resulting in 95% sterile and viable explants with minimal losses. Given the limited number of published studies focusing on sterilization in African violet tissue culture, our findings contribute valuable insights toward establishing a standardized disinfection procedure. Moreover, integrating surfactants and multi-step sterilization strategies can significantly enhance the success of micropropagation protocols for species with pubescent leaf surfaces. Future research should explore alternative sterilizing agents, such as nanomaterial-based disinfectants, and assess their compatibility with tissue regeneration efficiency to further improve contamination control in *S. ionantha* cultures.

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