UNIVERSITI TEKNOLOGI MARA

DEVELOPMENT AND OPTIMISATION OF A HYBRID SURFACE STERILISATION PROTOCOL FOR *IN-VITRO* CULTURE INITIATION FROM LEAF EXPLANTS OF *Capsicum frutescens* AND *Solanum lycopersicum*

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ABSTRACT

As global agriculture grapples with the dual crises of climate change and food insecurity, the demand for resilient, disease-free crops has never been greater. Amid shrinking resources and rising population pressures, Plant Tissue Culture (PTC) stands at the forefront of agricultural innovation, offering a precise, sterile method to mass-produce healthy plants for food, medicine, and conservation. Yet, despite its transformative potential, standard protocols are often repetitive, labour-intensive, and heavily reliant on chemical sterilants and contamination control agents, which risk promoting antimicrobial resistance (AMR) and limit scalability. Therefore, this study introduces a Hybrid surface-sterilisation protocol for *In-Vitro* Culture (HP-IVC) as an innovative and scalable solution to reduce contamination, physical labour, and callus yield inconsistency and address these limitations. Using two Solanaceae model species; *Capsicum frutescens* with waxy adaxial foliar (WAF) and *Solanum lycopersicum* with trichomes adaxial foliar (TAF), the research investigated four objectives: developing an IVC leaf sampling kit using hydrogen peroxide and vacuum-sealed bags; optimising semi-manual sonozonation through ozonation, ultrasonic cleaning, and surfactant washing; enhancing sterilisation through cutting phase order, silica beads, and high-frequency electromagnetic treatment (HFET); and comparing callus induction performance using minimal Plant Preservative Mixture (PPM) and hormone treatments. Thus, Stage 1 results showed that 15% hydrogen peroxide with vacuum-sealed bags reduced Gram-positive bacteria by 12% and preserved sample freshness for 3–5 days. Stage 2's sonozonation protocol increased explant survival by 33.33% with container "C" and power sweep settings, while Treatment 4 further reduced mechanical stress, prolonging survival to one month. Pre-cutting delayed contamination by ~27 days (*C. frutescens*) and ~25 days (*S. lycopersicum*), with silica beads improving survival by over 60%, and HFET lowering mortality to 20% across leaf types. In Stage 3, HP-IVC showed 98.33% (*C. frutescens*) and 98.07% (*S. lycopersicum*) callus induction using 1.0 g/L PPM, compared to <15% under standard conditions; both maintained ~90% callus growth, validating callus formation as a key indicator of protocol success. Operational time also improved: HP-IVC needed only 27.5 minutes for 60 explants and 82.5 minutes for 180, versus 25–30 and 90 minutes using standard IVC. Overall, HP-IVC sterilisation protocol demonstrated a practical and biologically efficient alternative to standard protocols by reducing potential AMR risks, minimising chemical usage, decreasing procedural repetition and labour fatigue, while consistently supporting high product yield, making it a sustainable tool for various plant research.

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CHAPTER 2
LITERATURE REVIEW

2.1 Plant Tissue Culture (PTC) and Its Industrial Relevance

Plant Tissue Culture (PTC) is a ground-breaking technique in plant biotechnology that enables the growth and regeneration of entire plants from small sections of plant tissue, known as explants, in a controlled and sterile environment of selective media (Cassells, 1991; Herman, 1996; Abbas & Rehman, 2021; Kushnarenko et al., 2022). This method has transformed the way plants are propagated, conserved, and genetically improved (Md Setamam et al., 2014), making it an essential tool in modern agriculture and plant science (Roca & Mroginski, 1991; Cassells, 2001; Alcala et al., 2020). By exploiting the natural capacity of plant cells to regenerate, PTC allows for the rapid production of genetically identical plants, including providing significant advantages in plant breeding, conservation, and genetic engineering (Murashige & Skoog, 1962; George & Sherrington, 1984; Skoog & Miller, 1957; Reed & Tanprasert, 1995; Md Setamam et al., 2014; Bettoni et al., 2019; Shetty & Narasimhan, 2021).

The origins of PTC date back to 1902, when a botanist, Gottlieb Haberlandt, first proposed the concept of growing plant cells under artificial conditions. Though his early experiments were unsuccessful, Haberlandt is widely regarded as the pioneer of plant tissue culture (Negi et al., 2024). His visionary work laid the foundation for future breakthroughs in plant science, however it was not until the mid-20th century that significant progress was made, particularly with the discovery of plant growth regulators (PGRs), such as auxins and cytokinins, which control plant cell division and differentiation (Skoog & Miller, 1957; Steward, 1958; Leggatt et al., 1994). Later, the development of the Murashige and Skoog (MS) medium in 1962 further advanced the research in PTC, providing a reliable nutrient base that remains a key component of plant tissue culture to this day (Murashige & Skoog, 1962; Negi et al., 2024).

At the heart of PTC is the concept known as cell totipotency, which refers to the ability of a single plant cell to regenerate into a complete plant under the right conditions (Steward, 1958; Longoria et al., 2022; Kushnarenko et al., 2022; Loyola-Vargas & Ochoa-Alejo, 2024; Negi et al., 2024). This principle underpins the flexibility and wide-ranging applications of PTC in which heavily relies on essential features of “an aseptic