Agricultural Sciences, 2025, 16(9), 1036-1046
https://www.scirp.org/journal/as
ISSN Online: 2156-8561
ISSN Print: 2156-8553

Exploring and Optimising the Potential of Ginger (*Zingiber officinale Rea*) Tissue Culture for Crop Conservation and Sustainable Agriculture in Samoa

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How to cite this paper: Vaurasi, V.D. (2025) Exploring and Optimising the Potential of Ginger (*Zingiber officinale Rea*) Tissue Culture for Crop Conservation and Sustainable Agriculture in Samoa. *Agricultural Sciences*, **16**, 1036-1046.
https://doi.org/10.4236/as.2025.169059

Received: January 15, 2025
Accepted: September 21, 2025
Published: September 24, 2025

Abstract

Tissue culture offers a promising solution for preserving ginger (*Zingiber officinale Rea*) genetic resources and promoting sustainable agricultural practices in Samoa. This study aimed to refine tissue culture techniques to enhance local ginger propagation, focusing on the use of organic additives and plant growth regulators for rapid multiplication and disease-free plant production. The process began with the cultivation of a single axillary bud on Murashige and Skoog (MS) basal medium, successfully generating a sterile and pathogen-free explant. The incorporation of 6-Benzylaminopurine (BAP) at 2.5 mg/L led to an increase to eight buds within 12 weeks. Over seven months, this methodology resulted in the production of more than 360 buds, highlighting the effectiveness of the medium formulation. Given ginger's susceptibility to fungal and bacterial contamination, obtaining sterile explants was critical for successful propagation. Sodium hypochlorite proved to be an effective and less toxic sterilization agent, ensuring clean plant materials and enabling an efficient scaling-up process. The findings demonstrate the potential of tissue culture to produce disease-free ginger planting stock, which is crucial for sustainable agriculture in Samoa. This research also underscores the importance of optimising media formulations, including the use of organic additives like coconut water as a natural substitute for commercial growth regulators such as BAP. While these results align with previous studies, there is room for improvement in sterilisation protocols and media composition. Future research should explore the scalability of these methods and further refine organic al-

ternatives to enhance propagation outcomes. This study provides valuable insights into the sustainable cultivation and conservation of ginger in Samoa, contributing to the broader goals of agricultural development and food security [1].

Keywords

Zingiber Officinale Rea, Ginger, Tissue Culture, Genetic Resource Conservation, 6-*Benzylaminopurine* (BAP), Sustainable Agriculture

1. Introduction

Ginger (*Zingiber officinale Rea*), a perennial herb widely grown as an annual crop in tropical regions, holds significant economic, agricultural, and cultural importance in Samoa. Known for its versatility, ginger plays a vital role in culinary, medicinal, and industrial applications. Despite its potential, ginger production in Samoa faces notable challenges, primarily due to the limited availability of disease-free planting materials [2] susceptible to soil-borne diseases, which limits the production of disease-free planting material.

To address these issues, tissue culture has emerged as a transformative approach to ginger propagation. This technique enables the mass production of disease-free planting materials, offering a solution [3] to the limitations of conventional methods, depending on several factors. Additionally, advancements in ginger tissue culture have explored the use of substitutes such as coconut water as an organic growth enhancer and important additive, as it was characterized to have cytokinin, zeatin, and kinetin [4] and can be used as a phytohormone [5] [6]. Similarly [7], silicon has gained attention for its potential benefits in ginger propagation, particularly in enhancing plant resistance to abiotic and biotic stress, improving structural integrity, and boosting overall plant vigour [1] and the potential to alleviate abiotic and biotic stress and enhance seed germination. By integrating innovative techniques like tissue culture and exploring the use of natural growth enhancers, Samoa's ginger industry can overcome current challenges and unlock its full potential, both economically and agriculturally.

2. Background and Methods

This research focuses on developing a reliable protocol for the in vitro propagation of ginger (*Zingiber officinale Rea*) using rhizomes as explants. The study investigates the effects of modifying *Murashige and Skoog (MS*) (Gamborg, Murashige *et al.* 1976) medium with organic additives, including coconut water, activated charcoal, and silicon. These supplements were chosen for their ability to enhance plant growth, reduce phenolic toxicity, and improve overall development. Coconut water acts as a natural source of plant growth regulators and removes certain auxin and cytokinin from the culture medium [7]. Silicon is known to strengthen physiological resilience and plays a crucial role in various physiological

processes by transmitting signals from the production site to its target cells. This research seeks to establish efficient tissue culture techniques for the rapid propagation of disease-free ginger plants, addressing the persistent shortage of high-quality planting material in Samoa. While the approach offers potential to strengthen sustainable cultivation and meet rising market demand, its effectiveness will ultimately depend on developing protocols that are not only technically reliable but also economically viable for smallholder farmers. The anticipated outcomes extend beyond improved yields, as a locally adapted and cost-effective propagation system could reduce dependency on imported seed material, enhance resilience against crop diseases, and contribute to long-term agricultural development.

3. Research Sites and Timeline

The study was conducted between November 2020 and 2023, with the *in vitro* phase carried out at the Scientific Research Organisation of Samoa and the nursery phase at the Plant Tissue Culture Laboratory, School of Agriculture and Food Technology, USP Alafua Campus, Samoa.

4. Explant Selection and Preparation

Ginger rhizomes were initially sourced from local supermarkets for their viable axillary buds. However, due to challenges in obtaining pathogen-free explants, healthy ginger plants were cultivated in a greenhouse, monitored for diseases, and reintroduced into tissue culture.

A backup strategy was implemented, in which explants were taken from 1 - 2-week-old seedlings grown under greenhouse conditions. Axillary buds were pruned and washed thoroughly under running water. Surface sterilisation involved sequential treatment with ethanol, sodium hypochlorite, and Tween 20, followed by rinsing with sterilised water to ensure cleanliness. Explants were then trimmed and inoculated under sterile conditions.

5. Media Preparation

MS basal media were prepared using stock solutions of macro- and micronutrients, supplemented with plant growth regulators (e.g., BAP, NAA), and organic additives such as coconut water, activated charcoal, and silicon. Sucrose (3%) was added as a carbon source, and the pH was adjusted to 5.6 - 5.8 before adding gelling agents. Media were autoclaved at 121˚C for 15 minutes, cooled, and aseptically transferred to culture jars for inoculation.

Sterile culture conditions were rigorously maintained throughout the experiments, with close monitoring for contamination. All media were autoclaved to ensure aseptic handling during inoculation and culture maintenance.

6. Literature Review

Ginger (*Zingiber officinale Rea*) is a perennial herb widely cultivated for its culinary, medicinal, and industrial applications. [8] Also, its uses have extended to

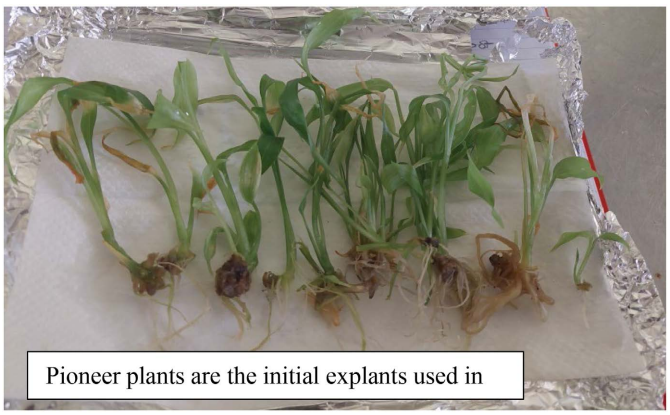
become useful as livestock [9]. However, its propagation is often constrained by the limited availability of disease-free planting materials, susceptibility to diseases, and the slow multiplication rate of traditional rhizome-based methods [10]. These challenges highlight the importance of innovative solutions, such as tissue culture, to improve ginger propagation, safeguard genetic resources, and support sustainable agriculture. The limited proliferation rate of ginger rhizomes has led to a shortage of planting material, much of which is vulnerable to disease.

7. Tissue Culture as a Propagation Tool

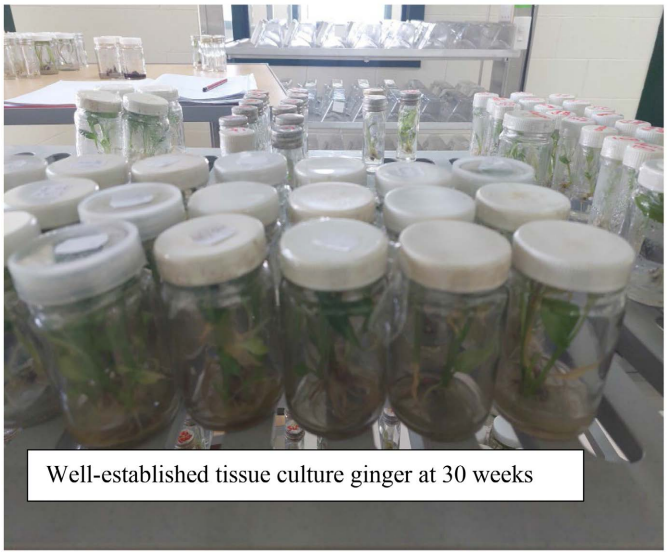
[11] Tissue culture has played a transformative role in modern agriculture by enabling the production of disease-free plants and has also found valuable applications in the pharmaceutical industry and in multiplying genetically disease-free plants, enhancing sustainable production [12] and ensuring homogeneity and high quality [13]. The use of plant regulators, such as 6-Benzylaminopurine (BAP) and Naphthaleneacetic Acid (NAA), has proven critical in optimizing shoot induction and multiplication. For example, BAP at concentrations of 2.5 - 3.0 mg/L has been found to significantly enhance bud initiation and proliferation in various ginger cultivars [14]. [10] These findings suggest that precise combinations of growth regulators can improve tissue culture outcomes and support the rapid multiplication of ginger plants.

8. Challenges and Opportunities

[15] Challenges have been observed with the use of tartrate salt compounds in culture media, as these formulations sometimes fail to solidify. Although tissue culture has proven highly effective for ginger propagation, optimising protocols for local conditions, such as those in Samoa, remains a significant challenge. Adjustments in media formulations and growth regulator combinations may be necessary to address regional environmental factors, genetic variability, and market demands [16]. Contamination continues to pose a fundamental obstacle in tissue culture, while issues of scalability and cost-effectiveness must also be considered to ensure the practical adoption of these techniques within small-scale and subsistence farming systems.



Pioneer plants are the initial explants used in



Well-established tissue culture ginger at 30 weeks

9. Analysis of Results

The results from this research indicate a highly successful rate of multiplication in ginger tissue culture. The study commenced with the cultivation of a single axillary bud on Murashige and Skoog (MS) basal medium, which was initially sterile and pathogen-free. Upon the addition of 6-Benzylaminopurine (BAP) at 2.5 mg/L, the multiplication rate was impressive, with the initial explant producing eight buds after 12 weeks. This marks a substantial increase from a single explant to eight shoots in just three months, demonstrating the effectiveness of the medium combination and the BAP treatment in promoting shoot proliferation.

Over the course of seven months, the multiplication process exhibited an exponential increase, with the number of buds rising to over 360. This growth represents a multiplication rate of over 45 times the original number of explants. The rapid multiplication underscores the efficacy of the chosen media and plant growth regulator combination, which facilitated the generation of a large number of disease-free ginger plants in a relatively short period.

10. Rate of Multiplication

To calculate the rate of multiplication, we can look at the increase from 8 buds at 12 weeks to over 360 buds after seven months. This demonstrates a **multiplication factor of 45 times** over the seven months.

If we break this down further:

- After 12 weeks, 1 explant produces 8 buds.
- By the end of 7 months (approximately 30 weeks), the total number of buds reaches more than 360.

Thus, the multiplication rate can be calculated as follows:

- Number of buds at 12 weeks = 8
- Number of buds at 30 weeks = 360

Using the formula for growth rate:

Multiplication rate = (Number of final buds/Number of initial buds)

Multiplication rate = 360/8 = 45 times

This rate is a strong indication that the tissue culture method used in this study can efficiently produce a large number of disease-free ginger plants, contributing significantly to the goal of scaling up ginger production for sustainable agriculture in Samoa. The actual experiment was conducted on December 13, 2022.

11. Discussion

The results of this study demonstrate the significant potential of tissue culture for the rapid multiplication and propagation of disease-free ginger. Starting with a single axillary bud, the process successfully resulted in a multiplication factor of 45 times within seven months, with over 360 buds produced. This success highlights the effectiveness of the media composition, which incorporated 6-Benzylaminopurine (BAP) and Murashige and Skoog (MS) basal medium, in combination with appropriate sterilisation and cultivation techniques. Previous studies have confirmed the use of BAP as a growth regulator that enhances shoot development and multiplication in ginger, demonstrating its reliability in tissue culture protocols [17].

A key finding of this study underscores the critical importance of starting the tissue culture process with clean, pathogen-free materials [2]. The use of sterile explants is fundamental in minimizing contamination, which remains a persistent challenge in ginger tissue culture due to its high susceptibility to fungal and bacterial pathogens. Previous research consistently demonstrates that contamination is one of the major constraints to successful propagation, reinforcing the necessity of maintaining strict aseptic conditions throughout the process. The findings of this study confirm that initiating cultures with sterile materials significantly reduces contamination risk and improves the likelihood of producing healthy, disease-free plantlets. However, it must also be acknowledged that contamination may still arise from sources such as latent infections or microbial carryover, indicating that sterilization at the initial stage, while essential, is not a complete safeguard. This highlights the need for more robust, multi-level contamination control strategies to strengthen the reliability of ginger tissue culture systems. [18]. The sterilization method employed—using sodium hypochlorite—proved effective in this study and is recommended for future work.

Sodium hypochlorite has long been employed as a disinfectant in plant tissue culture due to its accessibility, efficiency, and cost-effectiveness, making it a practical option for large-scale applications [19]. Its widespread adoption reflects its proven ability to reduce microbial contamination [20]. However, despite its effectiveness against bacteria, sodium hypochlorite is also known to exhibit cytotoxic effects on plant tissues and fibroblasts, raising concerns about potential damage to explant integrity [21]. While combining sterilisation with thorough washing can mitigate some of these adverse effects, this approach does not eliminate the risk of tissue injury or latent contamination. Therefore, although sodium hypo-

chlorite remains a valuable sterilising agent, its limitations highlight the need for more refined disinfection protocols that balance antimicrobial efficacy with the preservation of explant viability.

Once contamination-free explants were established, the culture medium was supplemented with 6-Benzylaminopurine (BAP), a commonly used cytokinin, to stimulate shoot proliferation. After 12 weeks, the addition of BAP resulted in a marked increase in axillary bud formation, with up to eight buds produced per explant. While this improvement underscores the importance of growth regulators in enhancing ginger propagation, it also raises critical considerations. Although BAP is widely reported to promote bud differentiation and shoot development across plant species [17], its effectiveness is highly dependent on concentration, explant type, and interaction with other hormones. Excessive reliance on BAP may risk physiological abnormalities, reduced rooting capacity, or somaclonal variation, which could compromise the long-term stability and field performance of propagated plants.

The results of this study confirm the role of BAP, in combination with MS medium, as an effective strategy for increasing shoot multiplication and generating healthy, disease-free plantlets. However, the broader implication is that successful propagation cannot be achieved through a single regulator alone. Optimisation of culture conditions—including fine-tuning growth regulator concentrations and evaluating synergistic effects with other cytokinins and auxins—is necessary to develop robust, scalable, and cost-effective protocols. Future work should critically examine not only the proliferation rates but also the genetic fidelity and physiological quality of the regenerated plants to ensure that tissue culture methods contribute reliably to sustainable ginger production systems.

The regulation of axillary bud formation is strongly influenced by hormonal signalling, particularly the interaction between cytokinins and auxins. Cytokinins generally stimulate bud initiation and outgrowth, while auxins exert an inhibitory effect, especially under conditions of apical dominance [22]. This antagonistic relationship underscores the complexity of hormonal control, as the balance between the two hormones shifts across developmental stages, leading to variability in axillary bud production and cell division cycles [23].

However, focusing solely on hormonal regulation oversimplifies the process, as environmental factors—such as light intensity, photoperiod, temperature, and nutrient availability—exert equally critical influences on bud initiation and growth. For instance, certain light regimes can enhance bud proliferation, while temperature fluctuations may either promote or suppress axillary development. This interplay between internal hormonal cues and external environmental conditions complicates efforts to standardize propagation protocols, since culture responses are not solely determined by exogenous hormone applications in MS media but also by environmental interactions.

In evaluating alternative sterilisation and propagation methods, several techniques were considered before ultimately being excluded based on their efficacy,

practicality, and alignment with the project's goals. The decision to exclude certain methods was influenced by factors such as their potential risks, limitations, and specific application challenges in the context of the study. Below is a discussion of the alternative methods and the reasoning for their exclusion, with particular reference to the propagation of ginger.

The exclusion of alternative sterilisation and propagation methods was largely based on their limitations in terms of practicality, cost, and efficacy. [24] Effective sterilisation methods eliminate barriers and encourage germination rate. [25] Mercuric chloride ($HgCl_2$) is commonly used as a sterilising agent to control contamination in micropropagation in fruit trees due to its powerful antimicrobial properties that increase the number of healthy plantlets [26] [27] and environmental sustainability. However, its use raises significant concerns regarding plant health and human well-being.

12. Enhancing Tissue Culture for Extensive Ginger Cultivation in Samoa

Scaling up tissue culture for ginger production in Samoa faces significant structural, financial, and technical barriers. Establishing and maintaining specialised facilities demand reliable infrastructure, yet rural areas often lack consistent electricity, water, and climate control. High capital costs for equipment and consumables, coupled with dependence on imported materials, further strain scalability compared to traditional propagation.

The shortage of skilled professionals in aseptic techniques and plant biotechnology compounds these constraints. Building technical capacity through continuous training for both laboratory staff and farmers is essential. Environmental instability—including frequent power outages, high humidity, and tropical temperature fluctuations—heightens contamination risks and complicates plant acclimatisation to field conditions. Ensuring pathogen-free donor material remains another critical challenge.

Economically, large-scale adoption requires strong market demand, stable supply chains, and supportive government incentives. Without these, the initial investment may outweigh returns, limiting farmer participation. However, public-private partnerships, local innovation in media and equipment sourcing, and comparative insights from tissue culture successes in other tropical crops could lower costs and enhance feasibility.

Despite the challenges, strategic investment in infrastructure, expertise, and institutional support could make tissue culture a viable pathway to sustainable ginger production in Samoa. By combining technical innovation with policy support and market development, it has the potential to deliver long-term gains in productivity, disease resistance, and agricultural resilience.

Recent advances in ginger tissue culture emphasize the development of alternative growth regulators, improved sterilization techniques, and hybrid approaches that integrate traditional and modern methods. These innovations demonstrate

the field's evolution and highlight opportunities to adapt global progress to Samoa's context. However, research on Samoa's indigenous ginger varieties remains limited, leaving a critical gap in understanding their response to tissue culture and their broader agricultural potential.

Economically, the adoption of tissue culture offers both promise and risk. Its benefits lie in the production of disease-free planting material, higher yields, reduced pesticide costs, and enhanced competitiveness in domestic and export markets. Yet, the method demands substantial upfront investment in infrastructure, specialised equipment, and imported consumables, making it costlier than conventional propagation. For smallholder farmers, this financial burden may hinder adoption unless offset by subsidies, credit access, or technical support.

Overall, tissue culture presents a transformative opportunity for Samoa's ginger sector, but its success depends on bridging research gaps, reducing operational costs, and ensuring systemic support. A robust cost-benefit analysis and targeted policies are essential to determine whether the approach can transition from an expensive innovation to a sustainable driver of productivity and resilience.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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