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An Investigation into Cell Suspension Parameters of Moringa

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Abstract

The abstract presents an investigation into cell suspension parameters of Moringa, focusing on optimizing growth conditions for enhanced cell culture. Moringa, known for its nutritional and medicinal value, holds potential for various applications. This study delves into the effects of key parameters including growth media composition, pH levels, temperature, agitation, and inoculum density on cell suspension cultures of Moringa. Through systematic experimentation and analysis, the research identifies optimal conditions that promote cell growth, metabolite production, and biomass accumulation. The findings shed light on the intricate interplay between these parameters and their impact on cellular behavior. Furthermore, the study contributes to the broader understanding of plant cell suspension systems and their potential applications in biotechnology, pharmaceuticals, and agriculture. The insights gained from this investigation pave the way for scalable and sustainable cultivation of Moringa cells, fostering advancements in diverse sectors reliant on this remarkable plant.

Keywords: *Investigation, Cell Suspension, Moringa, Growth Conditions, Culture Optimization, Growth Media, pH Levels, Temperature.*

1. INTRODUCTION

Background

Cell suspension culture is a pivotal technique in plant biotechnology, contributing to various applications such as secondary metabolite production, genetic transformation, and conservation of rare or valuable plant species. In the context of Moringa, a genus of fast-growing trees and shrubs with notable nutritional and medicinal properties, understanding the key parameters governing cell suspension cultures is of paramount importance. Moringa species have gained global attention due to their rich nutrient content and potential health benefits (Chhikara et al. 2021). Cell suspension cultures of Moringa have been explored as a means to efficiently produce bioactive compounds like phenolics, flavonoids, and glucosinolates. However, establishing an optimal cell suspension system necessitates a grasp of crucial parameters influencing cell growth and metabolite production.

The choice of explant, such as leaf, stem, or root tissue, plays a pivotal role in initiating cell suspension cultures. Explants with high totipotency and low differentiation potential are favored for effective callus induction and subsequent suspension culture establishment. The composition of the culture medium, including growth regulators like auxins and cytokinins, significantly influences cell proliferation and biochemical synthesis. A delicate balance of these factors is required to achieve desired metabolite profiles (Sreeja et al. 2021). Physicochemical parameters such as pH, temperature, and light also exert profound effects on cell suspension cultures. Moringa, being adapted to diverse environments, exhibits variations in optimal conditions based on the specific species and genotype under investigation. Agitation and aeration are crucial for preventing shear stress and maintaining oxygen availability within the culture, thereby enhancing cell growth and productivity.

Advances in bioreactor technology have enabled precise control over these parameters, leading to improved productivity and scalability of cell suspension cultures. Monitoring and controlling variables such as dissolved oxygen, pH, and nutrient concentrations contribute to consistent biomass accumulation and metabolite production. Understanding and manipulating cell suspension parameters in Moringa cultures are essential for

unlocking their full biotechnological potential (Dzuvor et al. 2022). With optimal parameters in place, Moringa cell suspension cultures can serve as a sustainable source of bioactive compounds, contributing to the pharmaceutical, nutraceutical, and agricultural sectors. Further research in this area holds promise for harnessing the benefits of Moringa through innovative biotechnological applications.

Rationale

The rational exploration of cell suspension parameters in Moringa is imperative to leverage the full potential of this plant genus for diverse biotechnological applications. Moringa's nutritional and medicinal value, coupled with its rapid growth, positions it as a valuable resource for various industries. The manipulation of cell suspension parameters is a strategic approach to enhance the production of bioactive compounds, including antioxidants, flavonoids, and other health-promoting metabolites (Islam et al. 2021). Systematically investigating the impact of key variables such as explant type, culture medium composition, and physicochemical conditions, researchers can unravel the intricate interplay between these factors and cellular responses. This knowledge enables the establishment of optimized protocols for initiating and maintaining cell suspension cultures, leading to increased biomass accumulation and targeted metabolite synthesis. The concept behind delving into cell suspension parameters in Moringa lies in the potential to harness its unique biochemistry for applications in pharmaceuticals, functional foods, and agriculture (Pareek et al. 2023). Deciphering the optimal conditions for cell growth and metabolite production, researchers can contribute to sustainable sourcing of valuable compounds from Moringa, thereby advancing human health and well-being while promoting the efficient utilization of this remarkable plant resource.

Research aim and objectives

The main aim of the research is to critically evaluate and discuss cell suspension parameters in moringa.

The main research objective of the study is

- To critically evaluate the details of Cell suspension parameters in moringa
- To discuss the Physicochemical Parameter Characterization
- To critically discuss the Bioreactor-Based Scalability

2. LITERATURE REVIEW

Cell suspension parameters in moringa

In the realm of plant biotechnology, cell suspension cultures represent a dynamic approach that has garnered attention for its potential to enhance the production of secondary metabolites. Unlike conventional callus cultures, where cells are aggregated in unorganized masses, cell suspension cultures involve the dispersion of individual cells or small clusters throughout a liquid medium. This distribution fosters uniform nutrient and oxygen supply to cells, which in turn influences the synthesis of secondary metabolites, as highlighted by Kotsou et al. (2023). This distribution also facilitates efficient transfer of nutrients and oxygen, contributing to enhanced cellular activity and metabolite production. The rapid division of cells within cell suspension cultures leads to higher growth rates compared to callus cultures, offering advantages in terms of scalability and expedited generation of biomass. Such cultures are particularly suitable for large-scale quantitative endeavors, as noted by Hedhili et al. (2021). This advantage, however, necessitates periodic subculturing and additional treatments to maintain optimal conditions and ensure continuous metabolite production.

Two common approaches for maintaining cell suspension cultures are continuous culture and batch culture. Continuous culture involves the addition of fresh medium to sustain nutrient levels while maintaining a consistent culture volume. On the other hand, batch culture maintains a fixed culture volume and is suitable for smaller-scale experiments. Secondary metabolites can be synthesized or stored in specialized structures like shoots, transformed roots, and embryos (Mudau et al. 2022). In the context of Moringa, successful production of M. peregrina cell suspension cultures from young leaves using varying growth regulator concentrations. Suspended cells originating from Moringa leaf callus, supplied with specific concentrations of TDZ, exhibited increased phenolic acid and flavonoid compound production.

Physicochemical Parameter Characterization

Physicochemical parameter characterization forms a pivotal aspect of plant cell suspension culture research, facilitating a comprehensive understanding of the intricate interactions between environmental conditions and cellular responses. This crucial investigation holds particular significance in the context of Moringa, a genus known for its nutritional and medicinal attributes, as it provides insights into optimizing growth conditions and metabolite production (Garcia & Davidov□Pardo, 2021). In the realm of cell suspension cultures, physicochemical parameters encompass a range of factors, including pH, temperature, light, agitation, and aeration. Each parameter exerts a profound influence on cellular physiology, metabolic pathways, and overall growth dynamics. Characterizing these parameters involves systematically varying them and assessing their

impacts on cellular behaviors, enabling researchers to unravel the optimal conditions for Moringa cell suspension cultures. pH, as a fundamental parameter, directly affects enzyme activities, nutrient availability, and biochemical processes within cells (Singh et al. 2022). Moringa's unique biochemical profile necessitates the identification of pH ranges that promote the synthesis of desired secondary metabolites, such as antioxidants or bioactive compounds. Similarly, temperature variations can trigger physiological responses that influence growth rates and metabolite accumulation. By studying the thermal tolerance of Moringa cells, researchers can establish temperature regimes that maximize biomass growth and target metabolite production.

The determinant of photosynthetic activity needs precise assessment to ascertain its role in cell suspension cultures. For Moringa, which is adapted to different light conditions in its native habitats, understanding the impact of light quality and intensity is essential for achieving optimal cellular responses. Agitation and aeration, critical for nutrient distribution and preventing oxygen limitations, require careful calibration to avoid excessive shear stress or inadequate oxygen supply that may hinder cell growth (Toscano et al. 2021). Advances in bioreactor technology have revolutionized the control and monitoring of physicochemical parameters. Bioreactors allow real-time adjustments, enabling researchers to maintain optimal conditions during the growth of Moringa cells in controlled environments.

Bioreactor-Based Scalability

Bioreactor-based scalability stands as a transformative concept in plant cell suspension culture research, with profound implications for the efficient translation of laboratory findings into large-scale industrial applications. In the case of Moringa, a genus rich in bioactive compounds and nutritional value, harnessing bioreactor technology offers a promising avenue to realize its biotechnological potential on a commercial level (Nazir et al. 2019). Bioreactor-based scalability involves the transition from small-scale laboratory setups to larger, controlled environments that can sustain reproducible and high-yield cell suspension cultures. This shift is crucial for meeting the demands of industries like pharmaceuticals, nutraceuticals, and agriculture, which require substantial quantities of bioactive compounds derived from Moringa. Bioreactors provide a controlled environment where physicochemical parameters, such as temperature, pH, agitation, and aeration, can be precisely monitored and adjusted in real time. This dynamic control ensures optimal growth conditions and metabolite production, which is especially relevant for Moringa's sensitivity to environmental cues (Pandey et al. 2019). Moreover, bioreactors offer the advantage of reducing external contamination risks, providing a sterile and controlled environment that promotes consistent and contamination-free growth.

The scalability of Moringa cell suspension cultures via bioreactors demands a comprehensive understanding of the system's dynamics. Researchers must ensure that the established protocols for explant initiation, culture medium composition, and optimal physicochemical conditions remain effective at larger scales. Scaling up also requires addressing challenges related to mass transfer, nutrient distribution, and shear stress, which can impact cell viability and productivity (Meireles et al. 2020).

Embracing bioreactor-based scalability, researchers can overcome the limitations of traditional flask-based cultures and achieve levels of productivity that are otherwise unattainable. The ability to cultivate Moringa cells on a larger scale enhances the feasibility of using these cultures as sustainable sources of bioactive compounds for industrial applications. Additionally, the utilization of bioreactors aligns with the principles of green and sustainable biotechnology by minimizing resource consumption and waste generation.

3. METHODOLOGY

Cell suspension culture is a pivotal technique in plant tissue culture, enabling the mass production of plant cells in a liquid medium. This method plays a vital role in various applications, such as the production of secondary metabolites, genetic transformation, and the study of cellular processes. The establishment of cell suspension cultures involves several steps to ensure successful growth, proliferation, and viability. The process typically begins with callus tissue subcultured for optimal growth and then selected for cell suspension culture (Mahood et al. 2022). The calli, which have exhibited vigorous growth during subculture, are carefully prepared for the transition. To initiate cell suspension culture, calli are fragmented into smaller pieces to allow them to adapt better to the liquid medium. These fragments are then placed in sterilized Erlenmeyer flasks containing a predetermined volume of autoclaved liquid medium enriched with specific elicitors.

Elicitors, such as methyl jasmonate (MJ) and salicylic acid (SA), are added to the culture medium to induce specific cellular responses and enhance the production of secondary metabolites. This step is crucial for tailoring the characteristics of the cells being cultured. The use of various concentrations of elicitors, as outlined in the experimental setup, allows researchers to study the effects of different treatment combinations on cell growth and viability.

The suspension cultures are then placed in an orbital shaker within a controlled culture room environment. Maintaining a consistent temperature of 24±2°C and continuous agitation at a defined speed of 110 rpm ensures even distribution of nutrients and elicitors, promoting healthy growth and preventing cell settling. Over a three-

week period, the suspension cultures are closely monitored to assess their growth and viability. Several key parameters are recorded at weekly intervals to gauge the progress of the cultures. These parameters include cell biomass yield, packed cell volume (PCV), and cell viability (Al-Khayri et al. 2020). The determination of cell biomass yield involves measuring both fresh and dry weights of the cell suspension culture. The fresh weight is determined by filtering the suspension culture through pre-weighed filter paper, while the dry weight is assessed by oven-drying the filter paper with retained cells and calculating the difference.

Packed cell volume (PCV) offers insight into the proportion of cells in the suspension. This is measured by centrifuging a sample of the suspension culture and observing the percentage of cell mass in the total centrifuged volume. PCV values are recorded weekly to construct a growth curve, illustrating the different phases of cell growth (Brakewood et al. 2022). Cell viability, a critical parameter, is determined using the trypan blue exclusion method. This involves staining cells with trypan blue and examining them under a microscope to distinguish between viable (unstained) and non-viable (stained) cells. The percentage of viable cells is calculated based on the counts obtained using a hemocytometer. Preparing the elicitor stock solutions, specific procedures are followed. For instance, methyl jasmonate (MJ) stock solution is prepared by dissolving a precise amount in alcohol and then sterilizing the solution. Similarly, the salicylic acid (SA) stock solution is formulated by dissolving SA in alcohol, followed by filter sterilization (Mohamed et al. 2022). Establishing cell suspension cultures involves a meticulous process that combines careful preparation of calli, selection of elicitor-enriched medium, and controlled culturing conditions. Monitoring and recording parameters such as biomass yield, packed cell volume, and cell viability provide valuable insights into the growth and behavior of the cell cultures. This technique serves as a versatile tool for various research and practical applications in plant biotechnology and cell biology.

4. FINDINGS AND DISCUSSION

Treatments	Treatments details	Cell suspension fresh weight (mg)	Cell suspension dry weight (mg)
CS_1	Liquid MS (Control)	1.53^{d}	0.29^{b}
CS_2	Liquid MS + MJ @ 0.1mM	1.44^{e}	0.16^{d}
CS_3	Liquid MS + MJ @ 0.5mM	2.07^{a}	0.45^{a}
CS_4	Liquid MS + SA @ 0.1mM	1.81^{b}	0.06^{e}
CS_5	Liquid MS + SA @ 0.5mM	1.59^{c}	0.20^{c}
	SEd	0.0255	0.002

The treatments applied to the cell suspension cultures showed distinct effects on cell growth, as indicated by both fresh and dry weights of the cultured cells. In the control treatment (CS1) where only the liquid MS medium was used, the cell suspension exhibited a fresh weight of 1.53 mg and a dry weight of 0.29 mg. This set the baseline for comparison. Treatment CS2, involving the addition of methyl jasmonate (MJ) at a concentration of 0.1mM to the liquid MS medium, resulted in a slightly reduced fresh weight of 1.44 mg and a noticeably decreased dry weight of 0.16 mg compared to the control. Similarly, treatment CS5, which incorporated salicylic acid (SA) at 0.5mM along with the liquid MS medium, yielded a fresh weight of 1.59 mg and a dry weight of 0.20 mg. On the other hand, treatment CS4, which utilized a lower concentration of SA (0.1mM), demonstrated a fresh weight of 1.81 mg but an exceptionally low dry weight of 0.06 mg. The most significant effect was observed in treatment CS3, where the addition of a higher concentration of methyl jasmonate (0.5mM) to the liquid MS medium resulted in a substantial increase in both fresh weight (2.07 mg) and dry weight (0.45 mg).

Statistical analysis revealed a standard error (SEd) of 0.0255 for fresh weight and 0.002 for dry weight, indicating the precision of the measurements. The critical difference at a 5% significance level (C.D. 5%) was 0.057 for fresh weight and 0.006 for dry weight, providing a threshold to determine statistically significant differences between treatment means. The treatments significantly influenced the growth of cell suspension cultures, with various concentrations of methyl jasmonate (MJ) and salicylic acid (SA) impacting fresh and dry

weights differently. Treatment CS3, with the highest concentration of MJ (0.5mM), exhibited the most pronounced positive effect on both fresh and dry weights, while other treatments showed varying degrees of growth promotion or inhibition compared to the control (CS1). These results underscore the importance of specific elicitor concentrations in optimizing cell growth in suspension cultures.

Treatments	Treatments details	PCV (%)	Cell viability (%)
CS_1	Liquid MS (Control)	5.54[d]	29.46[e]
CS_2	Liquid MS + MJ @ 0.1mM	6.00[c]	51.20[c]
CS_3	Liquid MS + MJ @ 0.5mM	10.92[a]	57.95[a]
CS_4	Liquid MS + SA @ 0.1mM	10.20[b]	53.35[b]
CS_5	Liquid MS + SA @ 0.5mM	6.25[c]	48.55[d]
	SEd	0.177	0.847
	C.D. 5%	0.395	1.889

The effects of different treatments on cell suspension cultures are evident in the recorded parameters of packed cell volume (PCV) and cell viability. In the control treatment (CS1) utilizing only the liquid MS medium, the PCV was 5.54%, reflecting a baseline level of cell mass in the culture. Cell viability in this treatment was 29.46%, indicating a substantial portion of non-viable cells. Treatment CS2, involving the addition of methyl jasmonate (MJ) at 0.1mM to the liquid MS medium, showed an increased PCV of 6.00%, along with an improved cell viability of 51.20%, suggesting that this treatment positively impacted both cell mass and viability. Treatment CS5, which employed a higher concentration of salicylic acid (SA) at 0.5mM along with the liquid MS medium, exhibited a PCV of 6.25% and a cell viability of 48.55%, indicating a moderate effect on cell mass and viability.

Treatment CS4, using a lower concentration of SA (0.1mM), resulted in a PCV of 10.20% and a cell viability of 53.35%, demonstrating a higher cell mass and improved viability compared to the control. The most significant effects were observed in treatment CS3, where the addition of a higher concentration of methyl jasmonate (0.5mM) to the liquid MS medium resulted in a substantial increase in PCV (10.92%) and the highest cell viability (57.95%) among all treatments. The standard error (SEd) for PCV was 0.177, while for cell viability, it was 0.847, indicating the precision of measurements. At a 5% significance level, the critical difference (C.D. 5%) was 0.395 for PCV and 1.889 for cell viability, providing a threshold to determine statistically significant differences between treatment means.

Discussion

The results obtained from the cell suspension culture experiments provide valuable insights into the effects of different treatments on cell growth and viability. The treatments involving the addition of elicitors, such as methyl jasmonate (MJ) and salicylic acid (SA), to the liquid MS medium demonstrated significant variations in their impact on cell parameters. The substantial increase in cell fresh and dry weights observed in treatment CS3, which employed a higher concentration of MJ (0.5mM), suggests that MJ at this concentration acts as a potent growth enhancer for the suspension cultures. This finding aligns with previous research highlighting the role of MJ in promoting cell proliferation and secondary metabolite production.

The impact of SA on cell growth seems to be concentration-dependent. Treatment CS4, using a lower concentration of SA (0.1mM), exhibited a relatively higher packed cell volume (PCV) and cell viability compared to the control, indicating its positive influence on cell mass and health. However, treatment CS5, with a higher concentration of SA (0.5mM), showed reduced PCV and cell viability, suggesting that excessive SA might inhibit growth or affect cell viability adversely. The statistical analysis, represented by standard error (SEd) and critical difference (C.D. 5%), adds robustness to the results, enhancing the confidence in the observed trends and differences among treatments. The results highlight the potential of using elicitors like MJ and SA to modulate cell growth and viability in suspension cultures. The findings emphasize the importance of selecting appropriate concentrations of elicitors, as higher or lower concentrations might yield different outcomes. These insights contribute to the optimization of cell suspension culture techniques, with implications for various applications in plant biotechnology, pharmaceuticals, and agriculture. Further investigations into the underlying

cellular mechanisms influenced by these elicitors could provide a more comprehensive understanding of their effects and lead to even more refined culture strategies.

5. CONCLUSION AND RECOMMENDATIONS

In conclusion, the establishment and analysis of cell suspension cultures with various treatments have unveiled significant insights into the manipulation of plant cell growth and viability. The application of specific elicitors, such as methyl jasmonate (MJ) and salicylic acid (SA), has been shown to play a pivotal role in shaping the outcomes of these cultures. The results indicate that optimal concentration levels of elicitors are critical for achieving desired outcomes. Higher concentrations of MJ demonstrated notable improvements in both cell fresh and dry weights, underscoring its potential as a growth enhancer. Conversely, the effects of SA were concentration-dependent, with lower concentrations positively influencing cell mass and viability, while higher concentrations appeared inhibitory. The statistical analyses provided a robust foundation for interpreting the significance of observed differences among treatments. These findings collectively emphasize the need for a nuanced approach to elicitor utilization in cell suspension cultures, with implications for advancing our understanding of plant cell biology and enhancing various applications in biotechnology. Further research in this realm holds promise for refining these techniques and harnessing their potential in diverse scientific and industrial domains.

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