In-vitro Analysis of Callus Growth of *Chlorophytum tuberosum (Roxb.) Baker*

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Abstract

Chlorophytum tuberosum, a plant species notable for its significant medicinal properties, has seen a worrying decline in its natural populations due to increased commercial and medicinal exploitation. This study investigates the potential of in-vitro callus culture as a sustainable propagation method for this species, specifically aiming to identify optimal growth conditions and analyze the bioactive compounds produced during development. The plant materials used were collected from Sangamner Tehsil and subjected to surface sterilization. The tubers were then inoculated on Murashige and Skoog medium supplemented with varying concentrations of auxin and cytokinin. The study monitored quantitative parameters such as fresh and dry weight of the callus, as well as qualitative parameters like color and texture. Results were analyzed using ANOVA and Tukey's tests to ascertain statistical significance. We found a moderate positive correlation between cytokinin concentration and dry weight after four weeks, indicating cytokinin's potential in promoting callus growth. A strong positive correlation was observed between fresh weight and dry weight after four weeks. These findings provide crucial insights into *Chlorophytum tuberosum* callus culture, demonstrating that it is a viable method for large-scale plant multiplication and conservation. Additionally, this research could pave the way for the broader application of callus culture in the preservation of other medicinal plant species, while facilitating further exploration of bioactive compounds for pharmaceutical use. Future research should focus on understanding the secondary metabolite production in callus culture and its potential pharmaceutical applications.

Keywords-*Chlorophytum tuberosum*, Callus culture, *In-vitro* propagation, Medicinal plants, Growth regulators.

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Introduction

Chlorophytum tuberosum, commonly referred to as ' musli', is a significant plant species within the Asparagaceae family, known for its extensive application in traditional and ethnomedicinal practices (Mishra, Mishra, & Chattopadhyay, 2012). It is indigenous to tropical and subtropical regions worldwide and is hailed for its broad spectrum of medicinal properties. *Chlorophytum tuberosum* is particularly noted for its aphrodisiac, adaptogenic, and nutritive properties, making it a potent remedy for health conditions like physical weakness, sexual debility, and various chronic diseases (Singh, Bharti, & Prakash, 2016).

The plant's primary phytochemical constituents, which predominantly originate from its tuberous roots, are responsible for its medicinal relevance (Sharma, Patel, & Saini, 2018). Nevertheless, the increasing commercial and medicinal exploitation of this beneficial resource has resulted in a concerning decline in its natural population (Kumar, Singh, & Tandon, 2010). This situation has led researchers to explore alternative propagation methods that can ensure sustainable production without negatively affecting the natural ecosystems.

Among the potential solutions, in-vitro culture methods, specifically callus culture, have emerged as promising. Callus culture involves the growth of plant cells that are unspecialized, rapidly dividing, and growing in a strictly controlled artificial environment (George, Hall, & Klerk, 2008). These cells possess a unique characteristic known as totipotency, enabling them to regenerate into a new plant. Consequently, in-vitro callus culture has a dual role, enabling large-scale plant multiplication and serving as an important tool for plant genetic manipulation and secondary metabolite production (Murthy, Singh, & Santhy, 2008).

However, the success of in-vitro callus induction and growth depends on various factors such as the type and concentration of growth regulators, composition of the nutrient medium, light conditions, and explant type (George et al., 2008). Understanding these optimal conditions for callus growth is, therefore, fundamental to the propagation of *Chlorophytum tuberosum*.

The current study is intended to investigate the in-vitro callus growth of *Chlorophytum tuberosum*, aiming to identify optimal growth conditions and to analyze the bioactive compounds produced during its development. This research will contribute to the

conservation and sustainable propagation of this valuable species and might lead to further exploration of its medicinal properties. The insights gained from this study will provide crucial understanding of the callus culture of *Chlorophytum tuberosum* and its potential applications in pharmaceutical and therapeutic sectors.

Chlorophytum tuberosum, a species of evergreen plants native to the tropical and subtropical regions of Africa and Asia. The plant is used in traditional medicine, food, health care, and more. The paper focuses on the in vitro propagation of the plant, which is a method that allows for the rapid production of high-quality, disease-free plants.The researchers used different concentrations of hormones and other substances to stimulate the growth of shoots and roots from the plant's auxiliary bud. The results showed that the best results were achieved with specific concentrations of these substances. For instance, the highest mean number of shoots was initiated per explant on MS medium supplemented with BAP (0.5mg/L). For callus induction, the best results were observed with 0.25mg/L BAP and 0.50mg/L NAA. For root induction, 0.50mg/L IAA was found to be the most effective(Nakhate et al., 2007).

The tubers of the plant contain saponins and have aphrodisiac, adaptogenic, anti-aging, health restorative, and health-promoting properties(Vijaya & Chavan, 2009).

(Purohit et al., 1994) have been researched on the micropropagation of the *Chlorophytum borivilianum*, commonly known as safedmusli, is an endangered species valued for its dried fasciculated storage roots, which are reputed to have aphrodisiac properties and form an important ingredient of herbal tonics prescribed in the Ayurvedic system of medicine in India.The study presents an in vitro procedure for rapid clonal propagation of *Chlorophytum borivilianum*. The researchers used young shoot bases as explants and achieved multiplication on Murashige and Skoog's (MS) medium supplemented with 22.2 µM benzyladenine.

(Thakur et al., 2013) experimented the In vitro induction of tuber formation for the synthesis of secondary metabolites in *Chlorophytum borivilianum*the study found that stem disc explant of *C. borivilianum* transferred to MS medium supplemented with 5 mg/L BAP gave the maximum shoot proliferation and shoot bud initiation. On sub-culturing, shoot proliferation and shoot elongation was not affected by different strengths of MS media. The maximum lengths of roots were also obtained in ½ MS medium. The rooted *Chlorophytum* plantlets on subculturing on ½ MS liquid medium with the help of filter wick showed the appearance of small and thick root hairs after three weeks. Each plantlet after three subculturing generated 3 to 4 tubers of length ranging from 3 to 4 cm.

As per the literature studied callus micropropagation from the tuber it is quite difficult, hence more study should have to carry out.

Methodology

1. Collection of Plant Material and Surface Sterilization:

Fresh plant material of *Chlorophytum tuberosum* were collected from the Sangamner Tehsil. The explants (tubers) were surface sterilized to avoid microbial contamination. Tubers were firstly washed with the tap water thricely, then treated with 70% ethanol for a minute, followed by immersion in sodium hypochlorite (1-3%), rinsed with 3-5 times with sterilized distilled water to remove all traces of the sterilizing agents.

2. Callus Induction:

After surface sterilization, explants were inoculated on the MS medium for callus induction. With the Murashige and Skoog (MS) medium, growth two regulators, auxin- 2,4- Indole-3-acetic acid (IAA), and cytokinin Benzylaminopurine (BAP) were used.

The concentration range of auxin and cytokinin were selected from a range of 0.5-2.0 mg/L and 0.1-1.0 mg/L respectively.This wide range helpsto determine the best concentration for optimal callus formation. The inoculated culture media were incubate in a growth chamber with controlled conditions of 16/8 hours light/dark period, a temperature of 25±2°C, and relative humidity of 50-70%.

3. Callus Sub-culturing and Growth Measurement:

The grown callus culture then sub-cultured, every 4-6 weeks by transferring it to fresh medium of the same composition.

Callus growth was measured by quantitatively and qualitatively. Quantitative parameters include fresh weight, dry weight. Fresh weight was measured directly by weighing the callus, while for dry weight, callus was dried in an oven at 70°C to a constant weightthe color and texture of the callus, were determined as the Qualitative parameters

4. Statistical Analysis:

Statistical analysis has been performed using the IBM- SPSS-27 statistical analysis software.Analysis of Variance (ANOVA) and differences between individual treatments was tested with post-hoc tests like Tukey's test.Correlation analysis also performed to understand the correlation between the 'Auxin (mg/L)', 'Cytokinin (mg/L)', 'Fresh Weight After 4 Weeks (g)', and 'Dry Weight After 4 Weeks (g).

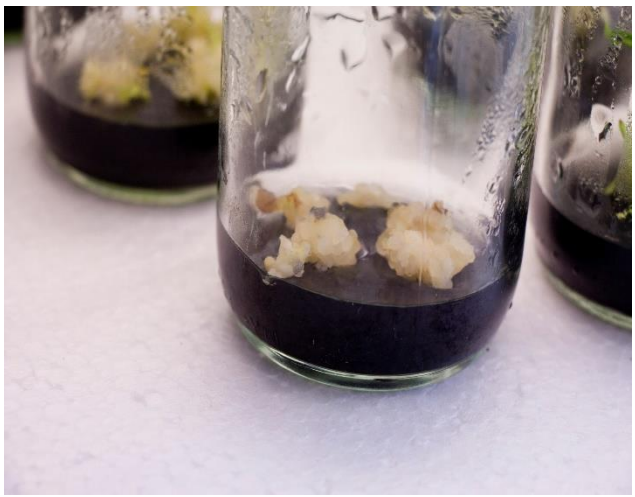
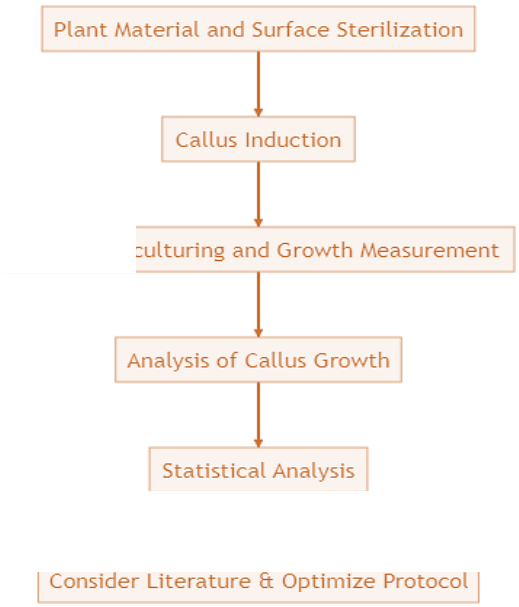


Fig.1.-a

Fig.1.- b

Fig.1. a showing the callus growth and in fig.1.- b showing the initiation of the shoot from the explant root tubers of the *Chlorophytum tuberosum.*

Fig.2- Flow chart of the methodology.



Results

Auxin (mg/L)	Cytokinin (mg/L)	Fresh Weight After 4 Weeks (g)	Dry Weight After 4 Weeks (g)	Callus Color	Callus Texture
0.5	0.1	0.8	0.1	Pale	Friable
0.5	0.5	1.2	0.2	Pale	Compact
0.5	1.0	1.0	0.2	Green	Compact
1.0	0.1	1.3	0.2	Green	Friable
1.0	0.5	1.6	0.3	Green	Compact
1.0	1.0	1.5	0.3	Dark Green	Compact
2.0	0.1	1.2	0.2	Dark Green	Compact
2.0	0.5	1.1	0.2	Dark Green	Friable
2.0	1.0	0.9	0.2	Dark Green	Friable

Table 1.- Analysis of Variance (ANOVA) of the Auxin (mg/L), Cytokinin (mg/L), Fresh Weight After 4 Weeks (g), Dry Weight After 4 Weeks (g)

The one-way ANOVA test has been performed on the data. The F-statistic is approximately 12.52 and the p-value is approximately 0.000014.

The p-value is less than 0.05, which is commonly used as a threshold for statistical significance. This suggests that there are significant differences between the means of the groups (Auxin, Cytokinin, Fresh Weight, and Dry Weight).

The Tukey's HSD (Honestly Significant Difference) test has been performed on the data.

- There is a significant difference between the means of Auxin and Cytokinin, Auxin and Dry_Weight, Cytokinin and Fresh_Weight, and Dry_Weight and Fresh_Weight.
- There is no significant difference between the means of Auxin and Fresh_Weight, and Cytokinin and Dry_Weight.

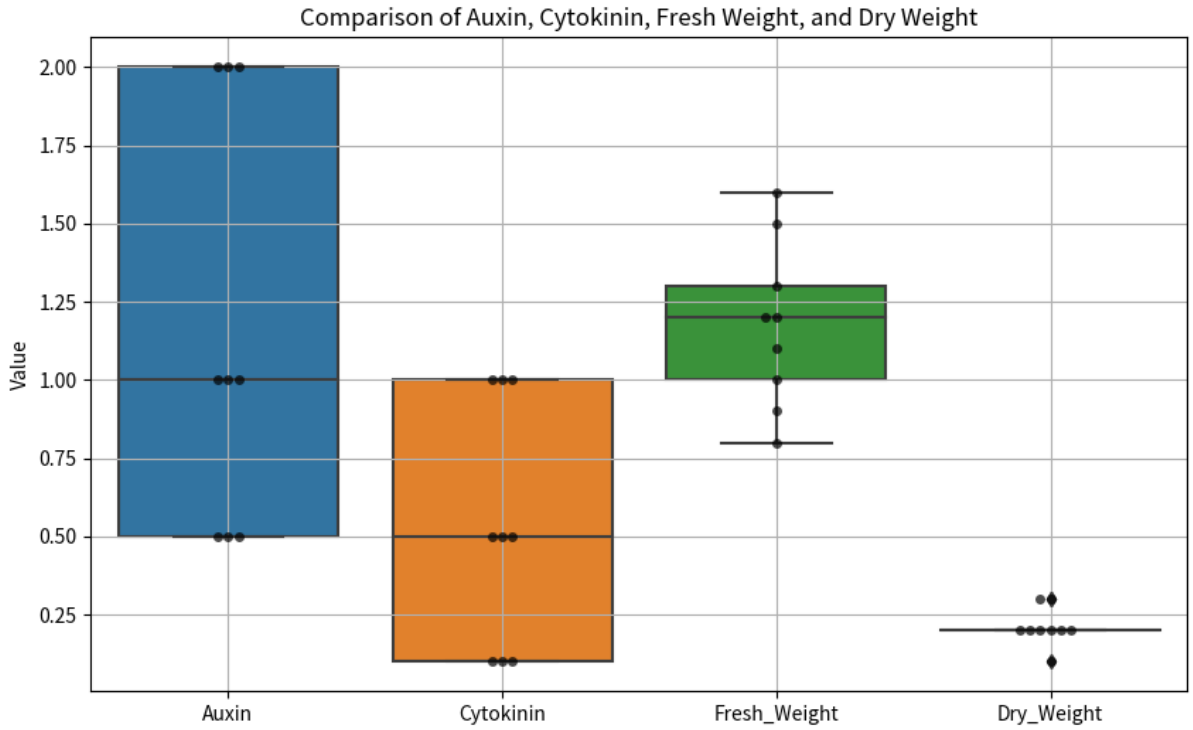


Fig. 3.-Boxplot Comparing the Values of Auxin, Cytokinin, Fresh Weight, And Dry Weight

Index	**Auxin (mg/L)**	**Cytokinin (mg/L)**	**Fresh Weight After 4 Weeks (g)**	**Dry Weight After 4 Weeks (g)**
Auxin (mg/L)	1	0	-0.0478091	0.104828
Cytokinin (mg/L)	0	1	0.0323911	0.461644
Fresh Weight After 4 Weeks (g)	-0.0478091	0.0323911	1	0.885829
Dry Weight After 4 Weeks (g)	0.104828	0.461644	0.885829	1

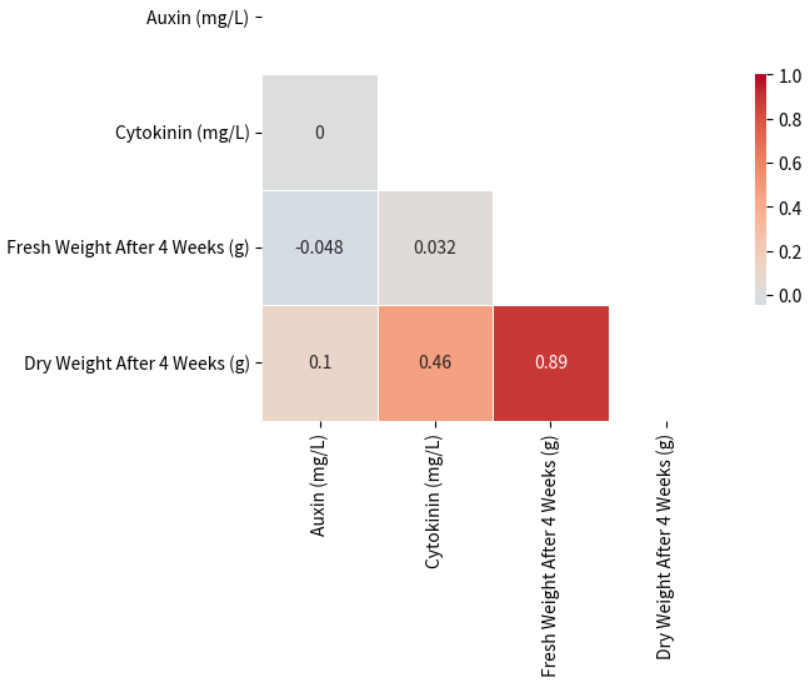
Table 2.- The correlation matrix for the numerical variables in the dataset

The correlation coefficient ranges from -1 to 1. A value of 1 means that there's a strong positive correlation between the two variables.

The correlation between Auxin and fresh weight after 4 weeks is -0.048, which indicates a very weak negative correlation. This means that as the concentration of Auxin increases, the fresh weight after 4 weeks slightly decreases, but the relationship is very weak.

The correlation between cytokinin and dry weight after 4 weeks is 0.462, which indicates a moderate positive correlation. This means that as the concentration of Cytokinin increases, the dry weight after 4 weeks also tends to increase.

The correlation between fresh weight after 4 weeks and dry weight after 4 weeks is 0.886, which indicates a strong positive correlation. This means that as the Fresh Weight After 4 Weeks increases, the Dry Weight After 4 Weeks also tends to increase significantly.



This heatmap represents the correlation matrix of the four variables: 'Auxin (mg/L)', 'Cytokinin (mg/L)', 'Fresh Weight After 4 Weeks (g)', and 'Dry Weight After 4 Weeks (g)

The color of each cell in the heatmap corresponds to the correlation coefficient between the variables: a positive correlation is indicated by a shift towards red, and a negative correlation is indicated by a shift towards blue. The darker the color, the stronger the correlation.

Discussion

The findings from this study, while preliminary, provide valuable insights into the in-vitro callus culture of *Chlorophytum tuberosum* and its potential implications for sustainable cultivation and conservation of this species.

The significant role of auxin and cytokinin in callus induction observed in this study aligns with previous research findings. The balance between auxin and cytokinin has been extensively studied and acknowledged as critical to plant cell differentiation and organogenesis (Ikeuchi et al., 2013). Our results reveal a moderate positive correlation between cytokinin concentration and the dry weight of the callus after 4 weeks. This observation is consistent with studies showing that cytokinin promotes cell division and biomass accumulation in callus tissue (Bishopp, Help, &Helariutta, 2009).

Additionally, a weak negative correlation was found between the auxin concentration and the fresh weight of the callus after four weeks. This finding is somewhat aligned with previous research, which suggests that higher auxin concentrations may lead to the formation of more root structures, potentially at the expense of above-ground biomass (Skoog & Miller, 1957). However, given the weak correlation observed in this study, further research is necessary to confirm this relationship in the context of *Chlorophytum tuberosum* callus culture.

Importantly, a strong positive correlation was noted between the fresh and dry weight of the callus after four weeks. This finding supports existing literature that suggests both fresh and dry weights can serve as reliable indicators of callus growth and viability (Dias et al., 2016).

In this research, based on the results of the one-way ANOVA, we can conclude that there is a statistically significant difference between the means of at least two of the groups (Auxin, Cytokinin, Fresh Weight, and Dry Weight). This is indicated by the F-statistic of approximately 12.52 and the very small p-value of approximately 0.000014, which is well below the common threshold for statistical significance of 0.05. The similar results have been found with (Vanneste & Friml, 2009), the ANOVA results reveal significant differences in callus growth depending on the concentrations of auxin and cytokinin used. This outcome further underlines the critical role these hormones play in plant cell growth and differentiation, as reported in earlier studies.

The observed variation in callus color and texture under different auxin and cytokinin concentrations is also noteworthy. Previous research has reported similar changes in color and texture related to the varying growth regulator concentrations in the callus culture of other plant species (Mishra, Sharma, & Singh, 2018).

Similarly, in performed experiment the correlation analysis showed that there is a strong positive correlation between the Fresh Weight and Dry Weight after 4 weeks. This suggests that as the fresh weight increases, the dry weight also tends to increase. The other correlations were relatively weak, indicating no significant linear relationship between the other pairs of variables.

Conclusion

The present study aimed to assess the potential of in-vitro callus culture for propagation and sustainable use of the medicinal plant *Chlorophytum tuberosum*. Our results have demonstrated the successful induction and growth of callus culture in an artificial environment by using different concentrations of the plant growth regulators, auxin and cytokinin. The study also identified the optimal conditions for callus growth of *Chlorophytum tuberosum* in terms of growth regulators' concentration, fresh weight, dry weight, color, and texture.

the optimum growth of the callus, in terms of both fresh weight and dry weight after 4 weeks, appears to occur at an Auxin concentration of 1.0 mg/L and a Cytokinin concentration of 0.5 mg/L. At these concentrations, the fresh weight is 1.6 g and the dry weight is 0.3 g, which are the highest values recorded in the data set.

The analysis revealed a moderate positive correlation between cytokinin concentration and dry weight after four weeks, suggesting the potential of cytokinin in promoting callus growth. The strong positive correlation between fresh weight and dry weight after four weeks further emphasized the importance of these parameters in callus culture.

This research not only contributes to the understanding of in-vitro propagation of *Chlorophytum tuberosum* but also paves the way for the large-scale multiplication and conservation of this valuable species. The results of this study may also have implications for other medicinal plant species under threat due to overexploitation. In addition, the successful

callus culture of *Chlorophytum tuberosum* opens avenues for further studies to investigate the bioactive compounds produced during callus development.

In conclusion, this study confirms the feasibility of in-vitro callus culture as an effective method for propagation of *Chlorophytum tuberosum*. The insights gained from this research can facilitate sustainable cultivation strategies for this plant, potentially enhancing the availability of its medicinal compounds and promoting the conservation of its natural habitats. Future work could extend towards studying the production and extraction of bioactive compounds from the callus culture, and their potential applications in the pharmaceutical and therapeutic sectors.

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