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Research Article

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Exploring morphogenic responses in tissue culture of diverse large cardamom (*Amomum subulatum*) varieties: A comparative study

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ABSTRACT
Large cardamom (*Amomum subulatum*) is one of Nepal's major cash crops. Tissue culture is an effective technique for enhancing its productivity and meeting market demand by facilitating disease-free plant production and rapid multiplication. This study aimed to investigate the morphogenetic responses of different large cardamom varieties under varying concentrations of Murashige and Skoog (MS) medium. The experiment was conducted at Machhapuchhre Plant Resource in Machhapuchhre-3, Ghachowk, using four varieties: Ramsey, Golsey, Varlangey, and Dambarsai. A Completely Randomised Design (CRD) with six replications was employed, resulting in four treatments. Data analysis was performed using Microsoft Excel, R-Studio, and Analysis of Variance (ANOVA) to assess the morphogenetic responses of the varieties. The results indicated that plant height, root length, and shoot length were not significantly affected, whereas plant weight, shoot number, root number, and leaf number exhibited significant differences. Among the varieties, Ramsey (Treatment 2) demonstrated the best morphogenetic response, while Dambarsai (Treatment 3) was the least responsive. The study highlights the potential of tissue culture in optimising large cardamom propagation by refining culture media, hormone concentrations, and environmental conditions. This method provides a reliable protocol for the mass production of disease-free, genetically uniform plants, offering a viable alternative to traditional propagation techniques, which are often slow and environmentally constrained. Furthermore, tissue culture plays a crucial role in conserving the genetic diversity of large cardamom, which is increasingly threatened by diseases, pests, and climate change. By ensuring a consistent supply of healthy plantlets, this approach can support sustainable cultivation and enhance commercial yields of large cardamom.
Keywords: Large Cardamom (*Amomum subulatum*), Micropropagation, Tissue culture, Morphogenic responses

INTRODUCTION

Large cardamom (*Amomum subulatum*), locally known as *Alaichi*, is one of Nepal's primary export commodities and among the most profitable cash crops. Although initially introduced to the Ilam district in 1865, its commercial cultivation began only in the late 1950s. Presently, large cardamom is cultivated across 51 districts, predominantly in the hilly and mountainous regions of eastern Nepal, while its production is gradually expanding towards the western regions (Shrestha, 2018). Belonging to the Zingiberaceae family, large cardamom is extensively cultivated in Nepal's eastern hills, central regions, and far western areas (Kumar et al., 2013). It is a cross-pollinated crop propagated primarily through seeds and suckers. However, seed germination is often weak, erratic, and may take over a year to germinate (Purseglove, 1975). Propagation methods for large cardamom include seeds, suckers, and tissue culture techniques. Seed propagation ensures virus-free plants, as viral infections do not spread through seeds. Conversely, vegetative propagation via suckers increases the risk of disease transmission from parent plants to new plantations. Tissue culture presents an efficient alternative, overcoming the limitations of poor seed germination and enabling the rapid mass production of disease-free seedlings. In vitro micropropagation has been recognised as a highly effective method for the large-scale production of healthy, high-yielding plants within a short timeframe (Bhojwani & Razdan, 1996). Given that large cardamom is a vital cash crop and a significant source of income for farmers in Nepal's eastern slopes, alternative propagation strategies are needed to address the challenges posed by fungal and viral infections, which

have contributed to declining cultivation areas (Poudel et al., 2018).

Nepal produces approximately 5000–6000 tonnes of large cardamom annually, with an average yield of 25–40 kg per ropani. Between mid-July and mid-February, Nepal's exports of large cardamom increased from NPR 2.2 billion to NPR 3.3 billion (Ekantipur, 2018). Approximately 99% of Nepal's large cardamom exports are destined for India, from where it is re-exported to Bangladesh, Pakistan, the Gulf countries, and other international markets. The average market price currently stands at NPR 1000 per kilogram. The key cardamom-producing districts—Taplejung, Panchthar, Ilam, and Sankhuwasabha contribute over 80% of the country's total production, with Taplejung having the largest cultivation area of 4500 hectares (MoALD, 2079/80).

Despite its economic importance, large cardamom production in Nepal faces several challenges. According to MoALD (2079/80), the total area under large cardamom cultivation is 18,175 hectares, with a productive area of 15,975 hectares, yielding 8674 metric tonnes at an average productivity of 0.54 metric tonnes per hectare. However, this yield is lower than expected due to the challenges associated with seed propagation, including delayed germination, susceptibility to diseases, and prolonged periods for flowering and fruiting (Kumar et al., 2012). Other propagation methods, such as sucker and rhizome propagation, also have limitations, particularly concerning disease transmission.

Tissue culture or micropropagation presents an effective alternative for commercial large cardamom production. Seed-grown plants take longer to develop and are highly vulnerable to diseases and environmental stresses, such as drought and waterlogging. In contrast, tissue culture-derived plants exhibit faster growth, higher multiplication rates, and enhanced resistance to biotic and abiotic stresses. Despite its potential, very limited research has been conducted on the micropropagation of large cardamom (Singh et al., 2010).

Large cardamom (*Amomum subulatum*), widely cultivated in the Himalayan region, is of substantial economic and medicinal value (Sharma et al., 2009). Given its importance in farmers' livelihoods and its applications in traditional medicine, optimising its propagation methods is crucial. Tissue culture provides a viable means for the large-scale production of uniform and disease-free plants, yet there is a lack of research on the tissue culture response of different large cardamom varieties. The technique holds promise for addressing supply chain challenges by enabling mass propagation of high-quality planting material (Bhojwani & Razdan, 1996). In rural hilly regions such as Kaski district, large cardamom is an essential cash crop. However, its slow growth cycle and vulnerability to diseases pose significant threats to its sustainability. While micropropagation has been successfully applied to various crops, studies on large cardamom remain scarce (Nadgauda et al., 1983). This research aims to fill the existing knowledge gap by investigating the micropropagation potential of different large cardamom varieties in the hilly region of Kaski district.

The study will evaluate the role of growth regulators in Murashige and Skoog (MS) culture medium in promoting the growth of meristematic tissues in large cardamom varieties such as Ramsey, Golsey, Varlangey, and Dambarsai. The research will identify the best-performing variety under controlled conditions, thereby contributing to the development of an efficient micropropagation protocol for large-scale commercial production. The primary objective of this study is to evaluate the morphogenetic responses of different large cardamom varieties to MS culture media under laboratory conditions. Specifically, it aims to identify the variety with the most desirable root growth in vitro, determine the best-performing variety for shoot growth in vitro, and evaluate the in vitro weight gain of different large cardamom varieties.

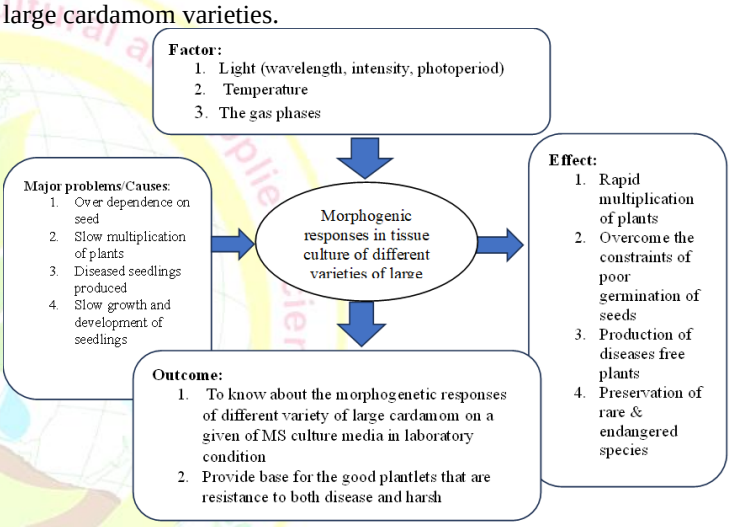

Figure 1. Conceptual Framework

MATERIALS AND METHODS

Description of the study area

This study was conducted at the Prime Minister Agriculture Modernisation Project (PMAMP) – Project Implementation Unit (PIU), Pokhara, Kaski, within the Cardamom Zone (28.3344° N, 83.9374° E). The site falls in a sub-tropical agro-climatic zone with temperatures ranging from 17 °C in January to 30 °C in May. November is the driest month, while July experiences the highest rainfall. The research was conducted at Machhapuchhre Plant Resource, Machhapuchhre-3, Ghachowk, from Chaitra 2080 to Ashad 2081. The site was selected for its suitability for controlled tissue culture experiments.

Treatments and experimental design

Variety

There were four varieties used in this research. They are:

Ramsey
Golsey
Varlangey
Dambars

Culture media

Preparation of 2000 ml of a concentration of large cardamom multiplication medium from the stock solution. The culture media for large cardamom micropropagation is MS media which is commonly used media for tissue culture with following ingredients:

Sucrose
BAP (Benzylaminopurine)
IBA (Indole 3-Butyric Acid)
Agar

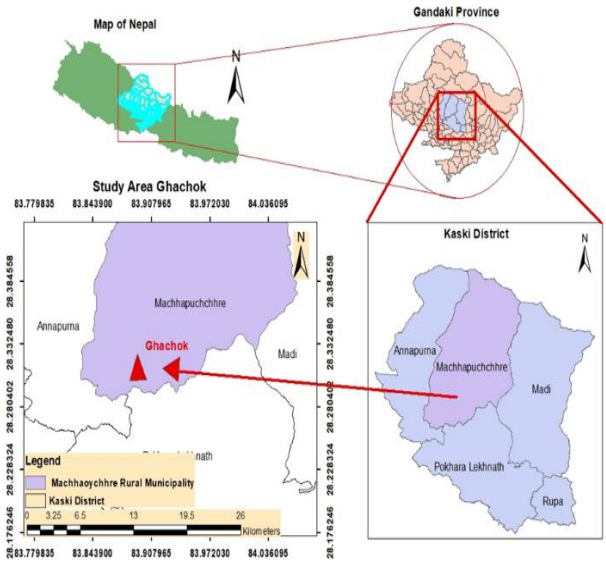

Figure 2. Map showing the location of the experimental field (Created by using Arc GIS software)

Experimental Design

The experiment design was carried out in one factor CRD design with 6 replications and 4 treatments. Each of the varieties was cultured in a MS basal medium with BAP 1mg/L and IBA 1mg/L.

R1T1	R6T3	R2T2	R2T1
R3T2	R6T4	R2T4	R6T1
R3T3	R2T3	R3T4	R4T2
R4T4	R6T2	R4T1	R5T1
R3T1	R1T2	R4T3	R5T2
R5T3	R5T4	R1T3	R1T4

Figure 3. A complete layout of experiment (Created Using MS Word).

Preparation of ex-plants

Ex-plants were taken directly from the field and then washed it for ½ hours in detergent water. Then, again after cutting the plant part, take rhizome part and taken it to the lab.

Tissue sterilization

Extracted rhizome buds of large cardamom
↓
Washed under running tap water to remove adherent soil for 30min
↓
Cut into 1.5cm -2cm length
↓
(Beaker) detergent Labolene solution for 10 min
↓
Rinse several times with running tap water
↓
Dipped and shake with 0.2% Bavistin & oil
↓
Tween 20 for 15-20 min in rotatory shaker
↓
Washed with running tap water clearly
↓
Rinsed with SDW (Sterilize Distilled Water)
↓
In LAF, 70% alcohol for 30sec and 1% NaOCl for 10 min
↓
Rinsed
↓
0.1% (w/v) HgCl2 for 5 min
↓
Rinsed 5 times with SDW
↓
Placed on sterilize blotting paper in Petri dish
↓
Cut & placed in MS media

Constituents of stock solution

MS-I (Macro Stock) 50X in 1000ml
NH_4NO_3 – 82.5g
KNO_3 – 95.0g
$MgSO_4.7H_2O$ – 18.5g
KH_2PO_4 – 8.5g
For 1 liter medium take 20 ml. It is stored at 2-4ºC for several weeks.

M-II (Calcium Stock) 100X in 500 ml ($CaCl_2.2H_2O$ - 22 g)
For 1 liter take 10 ml. It is stored at 2-4ºC for several weeks.

MS-III (Iron Stock) 100X in 500 ml
Na_2EDTA – 1.865g
$FeSO_4.7H_2O$ – 1.399g
First dissolve Na_2EDTA completely and then $FeSO_4.7H_2O$ is added.
For 1 liter take 10 ml. It is stored is refrigerator up to 1 year.

MS-IV (Micro Stock) 2000X in 500 ml
MnSO4.H2O – 16.9g
b) ZnSO4.7H2O – 8.6g
CuSO4.5H2O – 0.025g
COCl2.6H2O – 0.025g
KI – 0.83g
NaMoO4.2H2O -0.25g
H3BO3 – 6.2g
Sulphates are dissolved separately one by one is SDW & then boric acid.
For 1 liter take 0.5 ml. It is stored is refrigerator up to 1 year.

MS-V (Vitamin Stock)
Glycine – 0.2g
Nicotinic acid – 0.05g
Pyridoxin HCL – 0.05g
Thiamine HCL – 0.01g
Myo-inositol (added fresh) – 0.1g/L

For 1 liter take 2.5 ml. It is stored is refrigerator up to 2-3 month (-20ºC)

reparation of multiplication medium from stock solution (For 1L)
MS-I (50X) – 20ml
MS-II (100X) – 10ml
MS-III (100X) – 10ml
MS-IV (2000X) – 0.5ml
MS-V (400X) – 2.5ml
Inositol (100mg/L) – 0.1g
Sucrose – 30g
BAP (1mg/ml) – 1ml
IBA (1mg/ml) – 1ml
pH – 5.8
Agar – 7.5g

Temperature and Humidity

The optimum temperature and humidity were maintained in the growth room inside the laboratory. The optimum temperature is *25°C* and relative humidity is 70-80%.

Safety precautions in laboratory

Proper handwashing
Wear complete personal
Decontaminate the work surfaces before and after the experiment using a suitable disinfectant
Check all media and reagents prior to use
Properly dispose of all waste
Provide separate bottles of media for each cell line in cultivation
Follow the laboratory guideline

Shoot proliferation and elongation

Experiment was carried out to evaluate the axillary and adventitious shoot regeneration and elongation on basal MS medium. Observation on shoot induction and shoot length will be recorded every two weeks. Shoot proliferation efficiency of each of the varieties will be determined based on no. of shoot induced per ex-plant and shoot length, after 7-8 weeks of culture.

In vitro rooting

Healthy well developed young explants was transferred to the MS media for root induction. Observation on days to root induction, root length, root number, will be recorded after 7-8 weeks, and responses will be expressed as percent root induction, average root number per rooted shoot and root length per plantlets.

Parameters to be taken

***No. of leaves per plant*:** The number of leaves of each sample from each treatment were taken after 45 days of inoculation and the average was calculated.

***Root number*:** The root number of each sample from each treatment were taken after 45 days of inoculation and the average was calculated.

Shoot number: The shoot number of each sample from each treatment were taken after 45 days of inoculation and the average was calculated.

Root length: The root length of each sample from each treatment were taken after 45 days of inoculation and the average was calculated.

Shoot length: The shoot length of each sample from each treatment were taken after 45 days of inoculation and the average was calculated.

Plant height: The plant height of each sample from each treatment were taken after 45 days of inoculation and the average was calculated.

Plant weight: The plant weight of each sample from each treatment were taken after 45 days of inoculation and the average was calculated.

2.4 Data analysis technique

Computer software i.e. Microsoft excel and R-studio were used for analysis of collected data. Data was subjected to analysis of variance (ANOVA) to find out the significance of treatment effect.

RESULTS AND DISCUSSION

Various observations were made during the experiment on morphogenic responses. Statistical analysis revealed significant differences in leaf number, shoot number, root number, and plant weight among varieties, while shoot length, root length, plant height remained statistically similar (Table 1). At 45 DAI, the Ramsey variety exhibited the highest leaf number (23.5), while Golsey had the lowest (13.33). Shoot number was significantly different among varieties, with Ramsey having the maximum (12.00) and Golsey the minimum (5.500). Similarly, root number varied significantly, with Ramsey showing the highest (31.3333) and Dambarsai the lowest (16.500).

Plant weight also differed significantly, with Golsey having the highest weight (5.7667 g) and Dambarsai the lowest (2.3333 g). In contrast, no significant differences were observed in shoot length, root length, or plant height, indicating similar elongation trends across varieties. The maximum shoot length (6.6833 cm) was recorded in Varlangey, whereas Ramsey exhibited the lowest (5.6000 cm). Similarly, Varlangey had the longest root length (5.2833 cm), while Dambarsai had the shortest (4.4333 cm). The tallest plants (11.9667 cm) were recorded in Varlangey, while the shortest (10.3333 cm) were in Dambarsai.

Root and shoot number, leaf number, and plant weight responded more rapidly due to their dependence on cellular division and differentiation, whereas shoot length, root length, and plant height required longer periods to show variation (Thorpe, 1995). Morphogenic traits such as shoot and root number and plant weight are closely associated with cellular proliferation, which is more immediately influenced by tissue culture conditions. In contrast, elongation traits depend on cellular expansion, which may take longer to exhibit differences (De Klerk, 2002).

The lack of significant differences in shoot length among varieties suggests genetic predispositions related to elongation stability (Steeves & Sussex, 1989). Controlled conditions in tissue culture minimise environmental variability, possibly limiting observable differences in elongation. Plant growth regulators (PGRs), particularly auxins and cytokinins, play a crucial role in morphogenetic responses. Auxins promote root initiation, while cytokinins enhance shoot proliferation. However, elongation traits may be less sensitive to PGR concentrations compared to traits like shoot and root initiation or biomass accumulation (Bhojwani & Razdan, 1996).

Table 1. Morphogenic responses of different varieties of large cardamom

Treatment	Shoot Length (cm)	Root Length (cm)	Plant height (cm)	Shoot number	Root number	Plant weight (g)	Leaf number
1	2.5823 (6.6833)	2.2939 (5.2833)	3.4554 (11.9667)	2.637^{bc} (7.000)	4.2419^{bc} (18.1667)	2.2677^{a} (5.1963)	3.9843^{b} (16.000)
2	2.3450 (5.6000)	2.2210 (5.000)	3.2353 (10.6000)	3.4474^{a} (12.000)	5.5894^{a} (31.333)	2.3099^{a} (5.3482)	4.8250^{a} (23.500)
3	2.4278 (5.9000)	2.1039 (4.4333)	3.2131 (10.3333)	2.7920^{b} (7.8333)	4.0502^{c} (16.500)	1.5243^{b} (2.3333)	3.9487^{b} (15.6667)
4	2.4495 (6.0667)	2.1419 (4.6000)	3.2691 (10.7167)	2.3361^{c} (5.5000)	4.5596^{b} (20.8333)	2.3994^{a} (5.7667)	3.6389^{b} (13.3333)
LSD (0.05)	0.2857	0.2161	0.2933	0.3242	0.4189	0.1934	0.4712
SE_m (±)	0.0968	0.0732	0.0994	0.1099	0.1420	0.0655	0.1597
F-value	NS	NS	NS	0.001	0.001	0.001	0.001
CV %	9.6776	8.1939	7.3957	9.6049	7.5463	7.555	9.5455
Grand Mean	2.4512	2.1902	3.2932	2.8032	4.6103	2.1253	4.0992

*Note: Means in the column with same letter (s) in superscript indicate no significant difference between treatments at 0.05 level of significance based on LSD-Test; "***" = significant at p-value <0.001; LSD= Least significant difference; SE_m= Standard error of mean; CV= coefficient of variation; NS= non-significant, figures inside brackets were obtained after square root transformation of respective treatment means*

CONCLUSION

The results revealed significant differences in certain growth parameters, while others remained statistically similar. Ramsey exhibited the highest leaf number (23.500), shoot number (12.000), and root number (31.333), making it the most promising variety for tissue culture propagation. In contrast, Dambarsai consistently exhibited lower values in key traits, indicating its relatively poor response. Plant height, shoot length, and root length showed no significant variation among varieties, suggesting uniform elongation trends under controlled conditions. However, plant weight varied significantly, with Golsey recording the highest weight (5.7667 g), highlighting its potential for biomass accumulation. Tissue culture has demonstrated its potential as a viable alternative to traditional propagation methods, offering a pathway for producing genetically uniform, disease-free planting materials at a large scale. This approach can contribute to overcoming challenges related to low germination rates, disease susceptibility, and environmental constraints. Future research should focus on field transplantation, acclimatisation protocols, long-term yield assessments, and genetic modifications to enhance resilience against biotic and abiotic stresses. These advancements will further optimise large cardamom cultivation, ensuring sustainable production and higher economic returns for farmers.

CONFLICT OF INTEREST

The author here declares that there is no conflict of interest in the publication of this article.

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