Micropropagation of *Arisaema* spp. (*filiforme* and *brinchangense*): explant selection and surface sterilization insights

**Abstract.** *Arisaema filiforme* and *A. brinchangense* are perennial herbaceous plants (family Araceae) found distributed in mossy forest, Cameron Highlands, with an elevation of 1,900 meters above sea level (a.s.l). The unique inflorescence formation resembling cobra has given this plant the name Cobra lilies, and suitable to be planted as ornamental plants. In addition, it has been used traditionally as a herb. However, the population of these two species is very limited, only thriving in higher elevation and considered as an endangered. Therefore, realizing its potential in the future as one of the new ornamental plants and materials for the herb bioindustry, a micropropagation approach was employed to produce these species in mass production. Seeds, rhizomes, and petioles were used as the explant materials, cultivated onto Murashige and Skoog (MS) media supplemented with different concentrations (0, 0.5, 1, 2 mg L\(^{-1}\)) of 6-Benzylaminopurine (BAP).

The findings revealed rhizomes and seeds to be significant explants for micropropagation, where the survival rate for these two are more than 80%. Petioles had 0% of survivability after eight weeks of culture due to the fungi infection and tissue necrosis. This study provides an insight into explant selection, where different plant organs have different survival rates due to tissue mechanical strength. Also, optimum surface sterilization process is very critical in micropropagation to avoid the contamination of the culture and also necrotizing.

**1. Introduction**

*Arisaema* is the fourth largest genus out of 150 accepted genera of the Araceae family [1]. The genus *Arisaema* is a perennial monocotyledon herbaceous plant, also known as Cobra-lilies and Jack-in-the-Pulpit. The genus consists of approximately 207 species, with a range that stretches from Central and East Africa to Southeast Asia, via Yemen, Oman, Pakistan, and

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Arisaema species discovered include A. anomalum, A. filiforme, A. fimbriatum, A. lamiatum, A. roxburghii, A. scortechinii, A. wrayi, and A. brichangense.

In Sabah, the species’ spathe is green, whereas in Peninsular, the spathe is red and occasionally stained with green. A. brichangense is endemic to the Cameron Highlands in Peninsular Malaysia. The spathe resembles that of A. anomalum

Tissue culture methods in plant tissue culture are also affected by surface sterilization technique. In addition, tissue culture can serve as an essential method for overcoming the problem of infertility. Studies on the propagation of five Alocasia species, whereas, has reported on the novel micropropagation of A. filiforme. This resembles tissue culture of A. brichangense. This species is also protected by the Batu Gangan Permanent Forest Reserve. This is a new species, A. scortechinii. In addition, tissue culture can serve as an essential method for overcoming the problem of infertility.
2. Methodology

2.1 Plant material collection

The Mossy Forest Scientific Expedition was held in Cameron Highlands, Pahang, from 10 to 14 March 2023. During the survey, two species of Arisaema were found; *A. filiforme* and *A. brinchangense*, thriving in the montane forest at the altitude of 1800 to 2000 m above sea level (a.s.l). The identification of these species was done through references to pertinent articles and books, including works by [3]; [4]; [14], as well as through discussions with experts in Araceae, notably Peter C. Boyce. It flourishes on the moist forest floor covered with thick organic litter under deep to semi-shaded conditions. The collected live specimen of *A. filiforme* and *A. brinchangense* were carefully placed in sampling bags to preserve their freshness, moisture, and prevent damage. In-situ location pictures were taken for documentation purposes, as shown in Figure 1.

![Fig. 1](https://example.com/example.jpg)

**Fig. 1.** (Left) Map of the sampling area, whereas, (Right, above) Image of *A. filiforme* inflorescence and (Right, bottom) Image of *A. brinchangense* inflorescence

![Fig. 2](https://example.com/example2.jpg)

**Fig. 2.** The explants selected for micropropagation, (A) *A. filiforme* and (B) *A. brinchangense*, where: fr = inflorescence; rh = rhizome; pt = petioles
2.2 Experimental design

Several factors were considered when designing the experiment including the donor plant species, cell wall activity, and explant selection. The donor plant species, Arisaema filiforme, A. brichangense, A. filiforme, and A. sp., were randomly selected for the study.

Table 1

<table>
<thead>
<tr>
<th>Factor</th>
<th>Arisaema filiforme</th>
<th>Arisaema brichangense</th>
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<tbody>
<tr>
<td>Cell wall activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Explant selection</td>
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</tbody>
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2.3 Surface sterilization

Surface sterilization was carried out using 70% ethanol (EtOH) for 20 minutes, followed by three rinsings in sterile distilled water. The plant explants were then disinfected in 3% sodium hypochlorite (NaOCl) with one drop of Tween 20® solution for 15 minutes. The samples were then washed with tap water to remove debris before being immersed for 5 minutes in 70% EtOH.

2.4 Culture incubation & growth room conditions

The cultures were stored horizontally in a culture room with a temperature of 28 °C, photoperiod of 12 hours, and a white fluorescent tube as light source. For the donor plant species A. brichangense, the cultures were grown on media supplemented with different concentrations of Benzyaminopurine (BAP) for micropropagation of A. filiforme.

3. Result and discussion

The results of this study on the effects of surface sterilization, explant selection, and donor plant species on the success of tissue culture were reported. The condition of the explants was maintained to prevent contamination. As mentioned in section 2.1, the plant materials were collected from different habitats with mild and cold temperatures around 18 °C. Therefore, the growth conditions for the donor plant species were set at 22 °C.
To overcome the issue of tissue necrosis, it was observed that the smaller size of the explants should be chosen for surface sterilization. Increasing exposure times to hypochlorite (NaOCl) and Tween 20 should effectively eliminate fungal contamination. However, it was determined that the presence of the endophytic microorganism in the tissue is likely due to the varying tissue hardness and conductivity.

Contamination control was an important aspect of the study, where proper surface sterilization is critically important for the success of TC studies. To maintain sterile conditions, the size of the explants should be increased to approximately 0.5 cm × 0.5 cm, as conducted by [18]. Their study indicated that shorter exposure durations led to tissue death due to microbial contamination, while prolonged exposure durations resulted in death due to chemical damage. Therefore, sterilizing solutions are not suitable for all explants. Increasing exposure times is likely to elevate necrosis, consistent with the findings by [19].

Table 2. The percentage of survival rate, browning, and percentage of viability of A. brinchangense and A. filiforme seedlings in different explant treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival Rate (%)</th>
<th>Browning (%)</th>
<th>Viability (%)</th>
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<tr>
<td>A. brinchangense</td>
<td>80 ± 7.5</td>
<td>96.67 ± 0.5</td>
<td>73 ± 2.5</td>
</tr>
<tr>
<td>A. filiforme</td>
<td>90 ± 8.7</td>
<td>90.67 ± 0.6</td>
<td>80 ± 6.7</td>
</tr>
</tbody>
</table>

Note: The results indicate that A. brinchangense showed a higher survival rate compared to A. filiforme. Additionally, the browning effect was lower in A. brinchangense, while the viability was slightly higher in A. filiforme. These findings support the importance of choosing appropriate sterilization protocols for surface sterilization of explants. Throughout the culture process, no browning effect was detected. The media and the explants were cleaned due to the soft chyma cells, xylem, and phloem components predominantly composed of ground tissue, namely parenchyma.
The findings show, even after the eight weeks of the culture, no callus formation was detected, the seeds were not germinated and no other response of the explants was observed. It is important to acknowledge the limitations and challenges encountered during the study. Future research should address these limitations and explore modifications to enhance the efficiency of the micropropagation protocol. The potential applications of the developed protocol for the commercial production of *Arisaema* species should be further investigated.

4. Conclusion

As conclusion, the selection of rhizomes and seeds as the explants for TC is significant as both have a high survivability rate. The type of explants use, condition, and health status of donor plants combined with proper surface sterilization technique is critically important in ensuring the success in plant tissue culture. The establishment of these surface sterilization and plant cultivation techniques could lead to more in-depth research and a greater understanding of *Arisaema* in general. The findings will have implications for the conservation and cultivation of these plant species and contribute to the field of plant tissue culture.

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