Sterilization Method of *Etlingera Elatior* Explant on Tissue Culture

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**Abstract.** *Etlingera elatior* or in Sundanese known as honje is a type of traditional medicinal plant that is potential to be developed. Utilization of honje raw material as a medicinal plant is still harvested in nature without cultivation process. Traditionally honje is propagated through rhizomes with high infection rates because the rhizomes experience decay caused by bacteria of *Phytophthora* species. To overcome this problem, tissue culture as a conservation effort is needed to produce free pests and diseases seedlings. This study was aimed to find out explants that gave the best results on the sterilization of honje plants that would be used as planting material on cultivation with tissue culture. This research was conducted at Plant Tissue Culture Laboratory of Agrotechnology Dept. of UIN Sunan Gunung Djati Bandung. This study used two types of explants i.e. bud and bud base in the process of sterilization, each repeated 20 times. The results showed that the best explant on honje plant on sterilization was in the treatment of base of bud explant by showing the lowest contamination level with the number of live explant almost 88% and showed the beginning of shoot formation at age 3 DAI.

1. Introduction

Honje is one of the potential medicinal plants belongs to the family zingiberaceae which is commonly propagated using rhizomes. Naturally or conventionally honje propagation using rhizome often experiences rhizome decay caused by *Phytophthora* species [1]. To overcome the problem of honje cultivation, it is necessary to do conservation efforts through *in vitro* technique. One of the success factors in *in vitro* cultivation is the selection of parts to be explants or plant material.

Generally, explant originating from young tissue is the best explant because the tissue is still active divided like meristem tissue. In contrast, older tissues that tend to divide more slowly due to decrease of cell regeneration. According to cells in meristem tissue are generally stable because meiosis in meristem cells occurs simultaneously with continuous cell division, so excessive duplication of DNA can be avoided.

A free-contamination explant becomes the absolute requirement in *in vitro* propagation. According to Gunawan, each plant has different levels of contamination depends on the type of plant, the part of the plant used, the surface morphology, the growing environment, the season of explant taking and the
age of the plant [2]. Contamination is the most common problem in tissue culture, according to Onwubiko et al. there are generally four sources of contamination: on plants both externally and internally, culture media that is not well sterilized, environmental conditions of culture, and wrong ways of working [3]. Based on that, the determination of a sterilization procedure for each plant will be different because it has different characteristics.

2. Method
This research was conducted in Tissue Culture Laboratory, Agrotechnology Department, UIN Sunan Gunung Djati Bandung. The research method used was descriptive method which aimed to describe the situation in each stage of research based on facts that occurred or appeared. This sterilization stage used two kinds of explants namely bud part and base bud, cut with the size of 1-2cm and incubated in the dark room for 4 weeks.

The sterilizing agent used 2 g L\(^{-1}\) bactericide immersed for 60 minutes, then rinsed with aquades, soaked with 2 g fungicide for 90 minutes, then rinsed with aquades, soaked in Clorox 20% for 7 minutes, Clorox 10% for 10 minutes and Clorox 5% for 15 minutes. Then rinsed with aquades and soaked using 90% alcohol for 5 minutes. Soaking using aquades for the last 3x, then soaked using 1mL L\(^{-1}\) preservative mixture plant for 10 minutes repeated 2 times and finally rinsed with aquades. It was grown on MS medium by adding 5% sucrose and BAP 5mg L\(^{-1}\).

3. Results
Based on the results of research conducted shoot explants used produced contamination as much as 28% (Figure 2). This contamination first appeared on day 4 after initiation caused by fungi. Contamination caused by the fungus is characterized by the presence of hyphae around eksplan to cover the whole eksplan. Contamination by the fungus is characterized by the formation of mycelium that is the hyphae that forms the body of the fungus, whether it is white or gray.

The fungus that caused contamination was thought originated from the genus Mucor, characterized by white hyphae resembling white thread, there was sporangium like black dot and the growth of mycelium is very dense. Another contamination found in this bud was contamination caused by bacteria. This contamination was characterized by the presence of mucus or fluid colored white milk on the surface of the media or around explant.

![Figure 1](image)

**Figure 1.** (A) Contamination Fungus and (B) Contamination Bacteria.

Based on the research results, the percentage of contamination on bud base explants was lower than that of explants from bud ie 12% with live explant percentage 88%. Based on the observation, contamination by bacteria first appeared on 22 DAI marked with a milky white liquid.
4. Discussion
The shoot base explant used in this study showed a low percentage of contamination with shoot explants. This is in accordance with the research conducted by Chawla that explants from young tissue will be more responsive so that cell regeneration can take place quickly [4, 5]. Contamination either by mold or contamination by bacteria can be categorized as internal contamination or external contamination. Gunawan states that external contamination on the surface of explants occurs within a span of 48 hours, while internal contamination has a response of up to 30 days [6]. From the results of the experiment it can be stated that contamination by both fungi on shoot explants and base shoots is categorized by external contamination, while contamination caused by bacteria is called internal contamination because it occurs at 22HIS.

In general, contamination caused by bacteria or fungi is very difficult to control and becomes a major problem in the multiplication using tissue culture. Bacteria have a protective glycocalyx that is an external substance that is sticky and envelopes bacterial cells. Glycocalyx will protect the inside of the bacteria from the outside environment that is not beneficial such anti-microbial. Glycocalyx will be able to inhibit antimicrobial effectivity causing the stronger growth of bacteria.

5. Conclusion
The results showed that the best explant on honje plant on sterilization was in the treatment of base of bud explant by showing the lowest contamination level with the number of live explant almost 88% and showed the beginning of shoot formation at age 3 DAI.

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