

## Acclimatization of *Eurycoma longifolia* (Tongkat Ali) Plantlets to *Ex Vitro* Conditions

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### Abstract

*Eurycoma longifolia* is one of the famous herbal plants with great medicinal benefits. The plant which also known as Tongkat Ali is well-known for treating erectile dysfunction and as energy booster. Tongkat Ali plantlets were produced using *in vitro* propagation technique to cater the demand for planting material. The development of successful acclimatization technique is prerequisite for *in vitro* propagation method. Acclimatization is a step to prepare the plantlets to survive and grow in different environment compare to laboratory. In this study, an attempt was made to identify the best potting media to acclimatize Tongkat Ali using glass chamber. Rooted plantlets about 2-4 cm height were used and subsequently transferred to different potting media for acclimatization i.e jiffy 7, sand, baked soil, mixture of cocoa peat and sand, sand and top soil and lastly cocoa peat and baked soil. Plantlets grown in jiffy 7 showed 100% survival followed by the mixture of cocoa peat and baked soil with 94.45% of survival. The acclimatized plantlets were transplanted into polybag and maintained in the shadehouse condition.

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### 1. Introduction

*Eurycoma longifolia* is an herbal plant from Simaroubaceae family. The plant usually found in the tropical forest of Asean countries. All parts of the plant (root, stem, root bark, and leaf) are proved to be medicinal [6]. Due to its benefits, a lot of Tongkat Ali based products are sold in the market. The most common method of *E. longifolia* propagation is through seeds. However, like other woody species, propagation of Tongkat Ali through seed is difficult due to the unreliable flowering habit and quick loss of viability as well as low seed germination rate and slow growth [6]. Tissue culture technique producing Tongkat Ali plantlets will provide the solution to encounter the shortage of planting material.

Tissue culture plantlet using *in vitro* propagation technique using seeds, leaves, shoots or stems as explants and grew it into new plant with same criteria with the mother plant. The process will start from surface sterilization, shoot multiplication, *in vitro* or *ex vitro* rooting and lastly acclimatization before field planting. Tissue culture plantlets were developed within the culture vessels under low level of light, aseptic conditions, on a medium containing ample sugar and nutrients to allow heterotrophic growth and in an atmosphere with high level of humidity. These contribute a culture-induced phenotype that cannot survive the environmental conditions when directly placed in a greenhouse or field [2].

Based on Malaysia Meteorology department, Malaysia had an average temperature of 22-34 °C and low humidity [10]. High loss or damage of *in vitro*

raised plants can occur when transferred to *ex vitro* conditions because of the transfer shock [7]. Acclimatization is the only way for the plantlets to be able to survive in the natural environment. After acclimatization, the plantlets need to be hardened a little more before transplanted to open field.

In this study, the best media were determined to acclimatize Tongkat Ali plantlets using glass chamber. The ultimate success of *in vitro* propagation lies in the successful establishment of plants in the soil [9]. When shoots or plantlets are transplanted from culture room to greenhouse conditions they may desiccate or wilt rapidly and can die as a result of the changes in environment, unless substantial precautions are taken to accommodate them. In commercial micropropagation, this step is often the limiting factor [8].

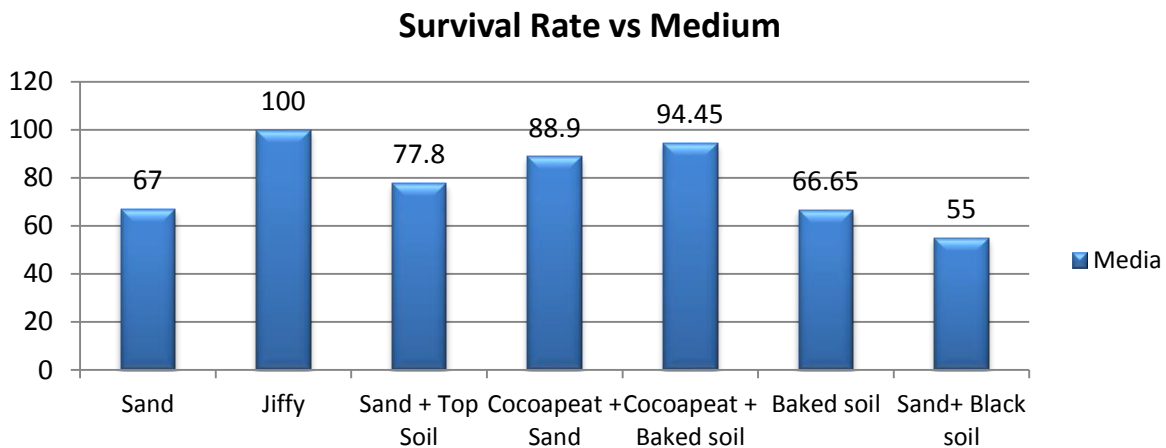
Usually the common method to acclimatized *in vitro* propagated plantlets was by using weaning chamber with scheduled misting system to maintain humidity. Throughout this study, plastic sealed glass chamber will be introduced as an alternative method to acclimatize Tongkat Ali plantlets.

## 2. Materials and Methods

*In vitro* rooted Tongkat Ali plantlets were used in this study. 2-4 cm plantlets were pulled out from culture media then washed under flowing tap water to remove adhered medium from the plantlets surface. The plantlets were then dipped into Thiram (fungicide) for few seconds to disinfect the plants. The plants then were planted in different medium with 3 replicates for each treatment. The plantlets were kept in a sealed glass chamber to maintain the humidity. The sealed was gradually open after 1 month and the plantlets were transferred into poly bag after 60 days. The plantlets will only be watered when the sealed opened. The plantlets were observed daily and the survival rates of plantlets were recorded.

## 3. Result and Discussion

In this study, several potting medium were used for Tongkat Ali acclimatization. After 1 month acclimatization in glass chamber, it was observed that plantlets using jiffy 7 as potting media achieved 100% survival rate. Followed by the mixture of cocoa peat and baked soil with 94.45 % survival rate. The lowest survival rate was observed in sand and black soil (55%).



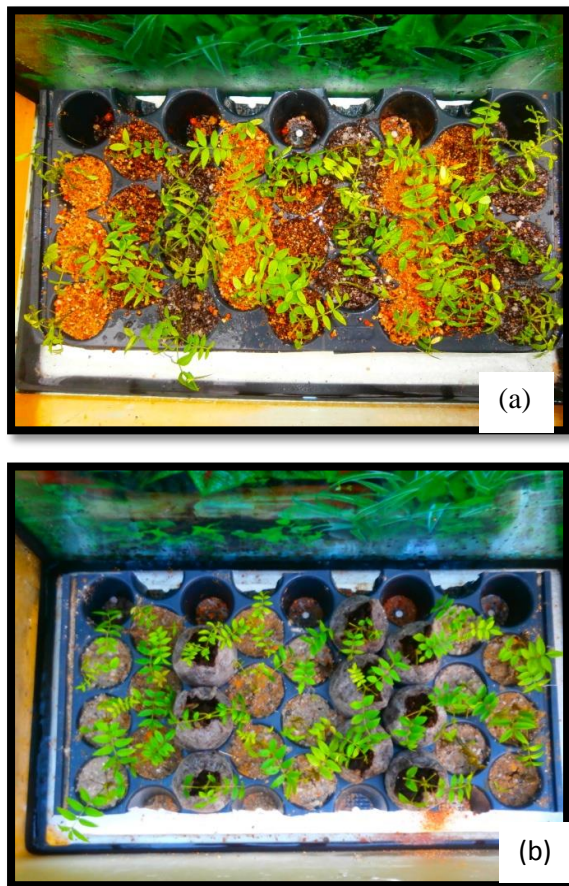
**Figure 1:** The survival rate of Tongkat Ali plantlets in different potting medium during acclimatization period

With minimal watering, potting medium containing peat showing good result compared to sand and other soils. Based on our observation, we could conclude that plantlets in sandy media need to be

watered more frequent to achieved higher survival rates.

We also observed that sealed glass chamber proved to be a better technique to acclimatized Tongkat Ali compared with acclimatization in weaning chamber

(Data not shown). Plantlets in the glass chamber need less water as the humidity and temperature were controlled inside the chamber. This process hardened the Tongkat Ali plantlets to adapt with changes of surrounding temperature and humidity.



**Figure 2:** (a, b) Tongkat Ali plantlets acclimatized in glass chamber using different potting media



**Figure 3:** Glass chamber used to acclimatized Tongkat Ali plantlets

#### 4. Conclusions

Jiffy is the best media for Tongkat Ali acclimatization using glass chamber.

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