UNIVERSITI TEKNOLOGI MARA

ESTABLISHMENT OF MICROPROPAGATION TECHNIQUE FOR *Hevea brasiliensis*

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ABSTRACT

Micropropagation offers many benefits to *Hevea* tree as this technique is capable to produce plant without seasonal interruption. In present research, the effect of different types of media and plant growth regulators on callus induction and shoot initiation from different explants of *Hevea brasiliensis* were investigated. The optimization of sterilization technique was conducted to reduce contamination and to obtain the most suitable media for callus induction and shoot regeneration on different explants of three *Hevea* clones; RRIM 3001, RRIM 2025 and PB 350. Callus were induced from the leaf and stem explants while shoot was initiated from the axillary buds. Leaf, stem and axillary bud explants were cultured on five different treatments which consist of three types of media (MS, WPM, DKW) supplemented with various combinations and concentrations of plant growth regulator namely α-naphtalene acetic acid (NAA), benzylaminopurine (BA), 2,4-dinitrophenylhydrazine (2,4-D), zeatin, sucrose and coconut water. The experiment was carried out at the laboratory of Genetic Transformation and Tissue Culture Programme (GTTC) Rubber Research Institute Malaysia, Malaysian Rubber Board Sungai Buloh, Selangor. Based on the results obtained, Procedure 3 was found the optimum sterilization procedure for all clones whereby increase in duration and concentration of sterilization solution help in removing contamination. It was also interesting to observe that Treatment 3, MS media supplemented with 0.5 mg L^{-1} BA, 0.5 mg L^{-1} 2,4-D, 0.5 mg L^{-1} Zeatin, 7% sucrose and 10% coconut water, gave significant effect on callus induction in both leaf and stem explants for all types of clones. Among the treatments, the highest callus diameter was observed in clone RRIM 3001 (stem : 2.34 cm, leaf : 1.95 cm), the highest plant survival rate was observed in clone RRIM 2025 (stem : 82%; leaf : 86%), and the fastest callus induction was observed in clone RRIM 2025 (stem : 9 day; leaf : 10 day). Different characteristics of callus such as friable, compact and yellowish were observed in all treatments. Meanwhile, Treatment 5 (DKW media + 0.5 mg L^{-1} BA, 0.1 mg L^{-1} NAA and 7% sucrose) was recorded as the best media for shoot induction. Among the treatments, the highest plant survival rate was observed in clone RRIM 3001 (42%) and the earliest shoot induction was observed on clone RRIM 3001 (28.8 day). For future study, investigation on the ability to produce root and acclimatization need to be conducted to assess the overall potential of *in vitro* *Hevea* seedling.

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CHAPTER ONE
INTRODUCTION

1.1 Background of Study

Hevea is widely recognized as one of the most important crops world wide due to the durability and flexibility of its latex. Latex is an amazing sustainable resource that can be harvested from the tree. The harvesting process is harmless to the tree and its economic life cycle is up to 32 years. Every year, demands for improved and high quality of rubber products are increasing. Thus, in order to fulfill the demands, biotechnology struggles have become more significant. Plant tissue culture is a tool in plant biotechnology which quantity and improvement quality of latex can be realized.

Efficient callus induction and shoot regeneration of *Hevea* tree under controlled environment will determine the competency of plant tissue culture technique in rubber industry development. *In vitro* regeneration of *Hevea* tree is also an important step in any genetic transformation and improvement protocol, because it provides source of starting materials, also known as explant to be used in genetic studies for crop improvement. Throughout the world, *in vitro* regeneration efforts in *Hevea* tree have been established using various types of explants such as embryo (Dickson *et al.*, 2011; Montoro *et al.*, 2010), anther (Quan *et al.*, 2012; Ying *et al.*, 2013; Nor Mayati, 2015) and vegetative tissues (Nor Mayati and Jamnah, 2014). The results obtained in these studies varied in different clones.

The importance of plant growth regulators (PGRs) in *in vitro* regeneration of *Hevea* tree has been discussed by many researchers (Nor Mayati and Jamnah, 2014; Min and Thu, 2001). A manipulation and determination of plant growth regulators in plant tissue culture could induce callogenesis, embryogenesis, organogenesis and rhizogenesis of plant tissues. Present study proposed with the aim to establish the micropropagation of three *Hevea* clones, which are RRIM 2025, RRIM 3001 and PB 350 using stem, leaf and axillary bud as explants. *In vitro* regeneration were conducted in various basal media including Murashige and Skoog (MS), Woody Plant Medium (WPM), Driver and Kuniyuki (DKW) and also supplemented with various combinations and concentrations of plant growth regulators, aiming to induce callus from leaf and stem explants as well as to identify the ability of direct shoot induction