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OPTIMIZATION ON MICROPROPAGATION FOR Typhonium flagelliforme (RODENT TUBER) IN DIFFERENT STRENGTH OF MS MEDIA, LIQUID CULTURE SYSTEM AND ANALYSIS OF TOTAL PHENOLIC CONTENT

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ABSTRACT

Tyhponium flagelliforme or rodent tuber is a medicinal herb which commercially used as anticancer supplement in Malaysia. This plant has limited sources due to their short life cycle, where the rhizome becomes dormant during dry season. Since *T.flagelliforme* biomass is needed for further research and commercialization, it is crucial to provide continuous production of this plant biomass. *In vitro* propagation through plant tissue culture (PTC) technique offers a tool for continuous and large scale production of T.flagelliforme. The present research aimed to optimize micropropagation of T.flagelliforme for their continuous supply and conservation. Changes in strength of MS media in solid and liquid media on growth, biomass and chlorophyll content of T.flagelliforme were evaluated. Then, the best treatment was used to investigate the effects of different liquid culture system on T.flagelliforme growth rate, biomass and chlorophyll content. The effects of different growth conditions on Total Phenolic Content (TPC) in T.flagelliforme leaves were also studied. The explants were cultured in different strength of MS media with and without addition of gelrite for 6 weeks with 15 replications. Then, T.flagelliforme shoot clumos were cultured in different liquid culture systems; Conventional Liquid Culture (CLC), Constant Immersion System (CIS) and Temporary Immersion Bioreactor System (TIBS) for 4 weeks with 6 replicates. All of the treatments were arranged in completely randomized design (CRD). Four leaves samples of *T.flagelliforme* were collected from *in vitro* Solid Culture Media (SCM), in vitro TIBS, in vivo and ex vitro leaves. Different types of culture media (liquid and solid media) have significant effects on the growth, FW, DW and chlorophyll (chl) content of in vitro grown T.flagelliforme. However, full- and halfstrength MS media has no significant effect ($P \ge 0.05$) on shoot height, shoots number, leaves number, roots number, FW and DW of T.flagelliforme. There was significance difference ($P \ge 0.05$) in chl a and chl b content of T.flagelliforme in full-, half- and quarter- strength MS. Full-strength MS liquid mediumwas the best treatment to increase shoots number (4.400 \pm 0.986), shoot height (24.467 \pm 5.273cm), leaves number (45.867 ± 11.389) , roots number (18.73 ± 4.01) , FW $(5.77 \pm 0.84g)$ and DW $(0.61 \pm 1.084g)$ 0.06g). The proposed liquid culture system using TIBS resulted maximum growth rate $(2.48 \pm 0.46 \text{ shoots/week}, 16.5 \pm 2.67 \text{ cm shoot height/week}, 8.75 \pm 1.7 \text{ leaves/week},$ $9.38 \pm 0.7 \text{ roots/week}$), biomass (5.99 $\pm 0.57 \text{ g FW}$ and 1.69 $\pm 0.45 \text{ g DW}$) and chl content system for T.flagelliforme. Maximum TPC was recorded in ex vitro leaves $(31.67 \pm 3.67 \text{ mg/g})$ followed by in vivo $(28 \pm 4.58 \text{ mg/g})$, in vitro TIBS $(27.33 \pm 0.67 \text{ mg/g})$ mg/g) and in vitro SCM (23.67 \pm 0.33 mg/g). Ex vitro T.flagelliforme cultured from plant tissue culture technique contain high phenolic content in their leaves and can also be considered a good source of medicinal remedies.

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CHAPTER ONE INTRODUCTION

1.1 Background of Study

There are growing interests of using plants as medicine in the whole globe. It is said that medicinal plants symbolize safety to human because they have better compatibility with the human body, less side effects and better cultural acceptability compared to synthetic drugs that are considered as unsafe to human and the environment (Kamboj, 2000; Singh, Kumar, Sharma and Singh, 2013). During long-term folk practice, a large number of medicinal plants have been screened and used for treating and preventing various diseases. The medicinal herbs such as *Typhonium flagelliforme* have been the chief source of natural compounds used for medicine.

Typhonium flagelliforme is a medicinal herb that belongs to the family Araceae (Arum). It is commonly known as 'rodent tuber' in English and 'keladi tikus' in Malay. Studies had proved that *T. flagelliforme* have anticancer (Lai, Mas, Nair, Majid, Mansor, and Navaratnam, 2008; Mohan, Abdulab, Abdelwahab, Al-Zubairi, Sukarid and Abdullah *et al.*, 2010), antibacterial (Mohan, Bustamam, Ibrahim, Al-Zubairi, and Aspollah, 2008), anti-inflammatory (Zhong, Zhou, Chen and Huang, 2001) and antioxidant properties (Mohan *et al.*, 2008). These medicinal properties of *T. flagelliforme* lead to the increasing demands of this plant biomass for further research and uses. Over-exploitation for pharmaceutical use will eventually depleted the species from its natural habitat.

Wild populations of *T. flagelliforme* are vulnerable to the indiscriminate commercial harvesting of this species. Conventional propagation of *T. flagelliforme* in the field is not sufficient to satisfy the increasing demand. This herb required specific growth conditions such as soft, damp and shady habitat (Dassanayake and Fosberg 1988). Putting further pressure on the species propagation could widen the gap between demand and supply. Therefore, the development of plant tissue culture method for *in vitro* propagation of *T. flagelliforme* and subsequently *ex vitro* acclimatization is needed to ensure a continuous supply of *T. flagelliforme* planting material and their sustainable use.