I. INTRODUCTION

1.1 Background

Curcuma sumatrana Miq., known as koenih rimbo, is a plant endemic to the island of Sumatera that has a vulnerable status based on the International Union for Conservation of Nature (IUCN) The Red List (Nurainas & Ardiyani, 2019). The distribution area of *C. sumatrana* is found in West Sumatera, mainly in Maninjau, Sianok, Lembah Anai, Kayu Tanam and Ulu Gadut of the Barisan Mountains (Ardiyani *et al.*, 2011). Recently, population of this species also found in Pesisir Selatan. The population of *C. sumatrana* is currently stable with around 1,00-1,000 individuals. However, populations of *C. sumatrana* are highly fragmented, and there is a continuous decline in adult individuals due to various factors, such as climate change, deforestation and human activities (Nurainas & Ardiyani, 2019).

C. sumatrana has a limited distribution but is widely recognized and extensively utilized by local communities, making conservation efforts and sustainable use a priority; it's status as a vulnerable species further underscores the urgency of developing population management strategies that integrate both conservation and balanced utilization aspects (Nurainas & Ardiyani, 2019; Rahayu, 2024). One technique to achieve this goal is toward plant tissue culture. This study was motivated by the need to increase the effectiveness of *in vitro* shoot multiplication on *C. sumatrana* by addition of TDZ either alone or in combination with BAP in media, as recommended by previous study (Arpita, 2024), this study

attempts to overcome the limitations of previous study. It is hoped that efficient and effective method for *in vitro* propagation of *C. sumatrana* can be obtained.

Idris (2024) conducted research on the growth of *C. sumatrana* shoots under *in vitro* conditions with modification of murashige and skoog basal media and glutamine supplementation. The results of this study found that MS basal media supplemented with 50 $mg.L^{-1}$ glutamine gave better results in shoot growth *in vitro*. Arpita (2024) also conducted research related to shoot multiplication using several concentrations of 6-benzylaminepurin (BAP) and AgNO3. This experiment has some weaknesses, especially that there is no optimum concentration of BAP gave better results in shoot multiplication. The results obtained showed that good growth and shoot multiplication was found at 2 $mg.L^{-1}$ BAP, even high shoot multiplication was found at 6 $mg.L^{-1}$ BAP. Therefore, optimizing shoot multiplication is essential to establish an efficient *in vitro* propagation protocol for this species.

Based on the results described above, it is necessary to apply cytokinin to *C. sumatrana* shoot multiplication need to be conducted, cytokinin is used in shoot multiplication treatment. Cytokinins are divided into adenine based and phenyl-urea based, 2-isopentyladenine (2Ip), 6-furfuryl-aminopurine (kinetin), N6 benzyladenine (BA), and 6-benzylaminopurine (BAP) from adenine based and Thidiazuron (TDZ) from phenyl-urea based (Jana *et al.*, 2013). Thidiazuron (TDZ) is mostly used in tissue culture due to its stability in the sterilization process (Aulia *et al.*, 2020; Dinani *et al.*, 2018). In general, the concentration range of TDZ use in shoot multiplication is 0,4 - 2 $mg.L^{-1}$ in the family of Araceae, Poaceae, Liliaceae, Musaceae, and Zingiberaceae (Dewir *et al.*, 2018).

Several previous studies has been examined the use of TDZ in shoot multiplication, especially in the Zingiberaceae family. Wannakrairoj & Wondyifraw, (2012) used TDZ in the range of 0 - 0,75 $mg.L^{-1}$ in shoot multiplication of *Amomum krervanh*. Karyanti *et al.*, (2021) using TDZ in the range of 0,1 - 1 $mg.L^{-1}$ for shoot multiplication and growth of *Zingiber officinale*. Murgayanti *et al.*, (2021) using TDZ at 1,5 $mg.L^{-1}$ for shoot multiplication of *Curcuma zedoria*. Zahid *et al.*, (2021) using TDZ at 1,10 $mg.L^{-1}$ for shoot multiplication of *Zingiber officinale*. Chuengpanya *et al.*, (2022) using TDZ in the range of 0,8 - 1,76 $mg.L^{-1}$ for shoot multiplication of *Hedychium longicornutum*. Vaze *et al.*, (2024) using TDZ in the range of 0,1 - 0,5 $mg.L^{-1}$ for shoot multiplication of *Curcuma pseudomonata*.

Inspite of single use, combination of TDZ with other cytokinins (BAP) are commonly used in the shoot multiplication. Lavakumaran & Seran (2014) use combination of 1 $mg.L^{-1}$ TDZ follow by 3 $mg.L^{-1}$ BAP accelerates the production of prominent and elongated shoot of *Aloe vera*. Nurmaningrum *et al.*, (2017) use combination of 0,9 $mg.L^{-1}$ BAP and 0,3 $mg.L^{-1}$ TDZ in shoot multiplication of *Medicago sativa*. Seo *et al.*, (2017) use combination of 1 $mg.L^{-1}$ BAP and 0,01 $mg.L^{-1}$ TDZ in shoot multiplication of *Hibiscus syriacus*. Gharari *et al.*, (2021) use combination of 1 $mg.L^{-1}$ BAP and 0,5 $mg.L^{-1}$ TDZ in shoot regeneration and multiplication of *Scutellaria bornmuelleri*. Nazihah *et al.*, (2023) use combination of 3 $mg.L^{-1}$ BAP and 0,01 $mg.L^{-1}$ TDZ for high shoot multiplication of *Musa* sp.

1.2. Formulation of Research Problems

The formulation of the research problem to be answered as follows :

1. What is the effect of TDZ concentration on shoot multiplication of *C. sumatrana* by using explant from shoot basal areas (meristematic area)?
2. What is the effect of BAP concentration on shoot multiplication of *C. sumatrana* by using explant from shoot basal areas (meristematic area)?
3. What is the interaction between the combination of TDZ and BAP on the shoot multiplication of *C. sumatrana* by using explant from shoot basal areas (meristematic area)?

1.3. Research Objectives

The objectives of the research are:

1. To observe the effect of TDZ concentration on shoot multiplication of *C. sumatrana* by using explant from shoot basal areas (meristematic area)
2. To observe the effect of BAP concentration on shoot multiplication of *C. sumatrana* by using explant from shoot basal areas (meristematic area)
3. To observe the interaction between the combination of TDZ and BAP on the shoot multiplication of *C. sumatrana* by using explant from shoot basal areas (meristematic area)

1.4. Research Benefits

This research is expected contribute to the development of more effective and efficient *in vitro* culture techniques for the multiplication of herbaceous plants, especially in the Zingiberaceae family. In addition, the results of this study are

expected to contribute to the conservation efforts of *C. sumatrana*, so that its existence in nature can be maintained.