1. INTRODUCTION

1.1 Background

Curcuma sumatrana Miq. or known by local people in West Sumatra as "Koenih Rimbo" in vername. This species is endemic to the island of Sumatra, Indonesia, which is currently reported to be only specifically distributed in 10 points between Lake Maninjau and Ulu Gadut in the Bukit Barisan Mountain, West Sumatra, spread across ten locations in the Bukit Barisan Mountain, especially between Lake Maninjau and Ulu Gadut, covering an area of approximately 2.217 km^2. *Curcuma sumatrana* is commonly used as a traditional remedy, with its leaves often boiled to treat skin itchiness. The plant's leaves are frequently utilized by the community to make "Pekasam" by wrapping the leaves around durian fruit flesh (Ardiyani *et al*., 2011).

Currently, its habitat is diminishing due to sustainable development activities, and the plant's presence is limited in the wild, with an estimated population of 100-1000 mature individuals (Ardiyani *et al*., 2011). According to the IUCN, *C. sumatrana* is currently classified as "*Vulnerable – VU*", therefore a suitable species propagation is needed. *C. sumatrana* is a seed-setting species that reproduces through seeds (Leong-Škorničková *et al*., 2010). However, this plant is commonly propagated vegetatively using rhizomes which takes quiet a long time to obtain new seedlings. To support conservation efforts for *C. sumatrana* and preserve its existence, mass plant propagation is necessary. One alternative that can be used for this purpose is tissue culture.

Root induction is a crucial step in plant tissue culture, aimed at completing the micropropagation process and ensuring successful plant adaptation in soil conditions (Hasnain *et al*., 2022). The primary purpose of root induction is to create a robust root system that allows the plant to absorb essential nutrients and water effectively (Amghar *et al*., 2021). This process involves the formation of adventitious roots from shoot explants, which is facilitated by the presence of vessels (sieve elements) in the stem and the creation of a temporary auxin maximum in xylem-adjusted cells (Pasternak & Steinmacher, 2024).

Root induction of *C. sumatrana* has not been reported, and this is a crucial stage in plant tissue culture for plant propagation. It enables the large-scale production of plantlets with consistent growth rates and root systems, suitable for further cultivation or secondary metabolite production (Noorrohmah, 2015). However, in critical situations, *ex situ* strategies play a vital role in the preservation of endangered plants and offer some scopes of saving wild resources against the loss of their natural habitats (Ayuso *et al*., 2019). Root induction is a vital step in *ex situ* conservation of rare or endemic species, plantlets that form good roots have a high survival rate during the acclimatization period in the greenhouse (Gianguzzi *et al*., 2023). A study on the *ex-situ* conservation of the endangered medicinal plant *Andrographis paniculata* developed a well-established root system within 15 days and were successfully acclimatized in the greenhouse (Monika *et al*., 2022).

The success of root induction is influenced by several factors, such as the plant species, the culture medium composition, and the application of plant growth regulators (Lestari *et al*., 2023). The studies on root induction in *Curcuma* highlight

the importance of plant growth regulators in stimulating cell division and differentiation, ultimately leading to the development of roots (Phakpaknam *et al*., 2023). The optimal concentrations of these regulators can vary depending on the specific *Curcuma* species (Sunitibala *et al*., 2001).

Media in root induction act as a nutrient supply by delivering essential substances required for plant metabolic processes (Indah & Ermavitalini, 2013). Half-strength MS medium is considered the best medium for root induction based on several studies. The effectiveness of this medium has been consistently demonstrated across various plant species. Highest root induction in *Catharanthus roseus* (Bakrudeen *et al*., 2011) was observed on half-strength MS basal medium.

Furthermore, similar results were observed in *Artocarpus altilis* (Noorrohmah, 2015), where the highest number of roots and root length were achieved. A 100% root induction rate, with a maximum of 4.2 roots per shoot and a maximum root length of 1.72 cm, was achieved on half-strength MS medium (Kumar *et al*., 2013). Another study reported the highest root induction rate (96.7%), with an average of 15.2 roots per shoot and a mean root length of 8.3 cm, on the same medium (Lee *et al*., 2020). These findings highlight the reliability of half-strength MS medium in promoting efficient root formation during plant tissue culture.

Root induction was highly influenced by the types and concentration of auxin that used (Khan *et al*., 2009). Naphthalene Acetic Acid (NAA) is a synthetic auxin, commonly used at relatively low concentration to elicit auxin type responses in cell growth, cell division, rooting, and fruit setting. Sun & Hong (2010). The adventitious

root production was increased rapidly at lower NAA concentration, while the number of roots was decreased at higher concentration (Khan & Bi, 2012).

Studies in *Curcuma* root induction has been conducted by Shahinozzaman *et al*. (2013) investigate the *in vitro* propagation of *C. zedoaria*, a valuable medicinal plant, using rhizome buds explants. For root induction, PGR's that used are NAA and IBA for single use (0.0-2.0 mg L^{-1}), best treatment of this research showed that 1.0 mg L^{-1} NAA produces the highest number of roots and the longest roots compared to the use of auxin d Indole-3-butyric acid (IBA). Another study by Jena *et al.* (2020) focused on the rapid plant regeneration in industrially important *C. pseudomontana* using rhizome buds. For root induction, PGR's that used are NAA, IAA and IBA for single use (0.0-1.5 mg L^{-1}), best treatment of this research showed that 0.5 mg L^{-1} NAA produces the highest number of roots and the longest roots compared to the other auxin. On the other hand, studies regarding root induction in *C. sumatrana* haven't been reported before, especially using planting explants in the form of shoots planted in media supplemented with NAA. Another study of root induction of *Zingiberaceae* family NAA at a concentration of 1.4 mg L-1 has been proven most effective in increasing the number of roots (Zahid *et al*., 2021).

Root induction is a critical step in *in vitro* propagation, as it significantly enhances the survival rate of plantlets during the acclimatization stage (Hardjo *et al*., 2023). Problems that often arise during acclimatization process is poor root quality can result in a low survival rate due to the inability of the roots to absorb water and nutrients from the *in vitro* environment (Mosoh *et al*., 2024). Root induction is an important to

ensuring that the plant can grow well after acclimatization (Yunita *et al*., 2016), especially for endangered plant species.

Parma (2024) carried out acclimatization *C. sumatrana* from the results of a combination of light intensity and sucrose concentration. This research states that the survival percentage of *C. sumatrana* is above 91%, so it is assumed that acclimatized plantlets can adapt to the acclimatization environment provided. Based on the description above, research regarding the effectiveness of NAA in root induction *C. sumatrana in vitro* needs to be carried out to increase the success of *C. sumatrana* propagation.

1.2 Formulation of Research Problems

Based on the description above, the problem formulation is as follows:

1. What is the effect of different strength of MS basal medium for root induction in *C. sumatrana in vitro*?
2. What is the effect of different concentration of NAA for root induction in *C. sumatrana in vitro*?
3. What is the effect of interaction between the different strength of MS basal medium and concentration of NAA for root induction of *C. sumatrana in vitro*?
4. How many percentages of plantlet survive after acclimatization stage?

1.3 Research Objectives

The objectives of the research are:

1. To observe the effect of different strength of MS basal medium for root induction in *C. sumatrana in vitro*.

2. To observe the effect of different concentration of NAA for the root induction in *C. sumatrana in vitro*.
3. To observe the effect of interaction between the different strength of MS basal medium and concentration of NAA for root induction in *C. sumatrana in vitro.*
4. To know the percentages of plantlet survival rate after acclimatization stage resulted from *in vitro* root induction.

1.4 Research Benefits

The benefits obtained from this research include acquiring scientific information and insights into *in vitro* root induction of *C. sumatrana*. The information obtained can support the successful implementation of micropropagation for *C. sumatrana* as a part of *ex situ* conservation efforts of Indonesian endemic species.