CHAPTER I. INTRODUCTION

1.1. Background

Dendrobium mussauense Omerod is a rare epiphytic orchid and endemic species distributed in New Guinea. *D. mussauense* has gained popularity due to its beautiful flowers with faint yellow colour, the white lip with purple veins, and the flowers widely opening (Puccio, 2022). *D. mussauense* can also be used in breeding programs to produce floriculturally important hybrids with attractive combinations of shape, colour, and fragrance (Pyati, 2020). The diversity of *D. mussauense* is under threat, and the IUCN (2018) categorized this species as vulnerable. *D. mussauense* populations are declining due to illegal hunting, unregulated collection, climate change and habitat destruction. Therefore, a conservation program for *D. mussauense* must be urgently considered.

Somatic embryogenesis is commonly used for clonal propagation of a wide variety of plant species (Moradi *et al*., 2017). Somatic embryogenesis (SE) is a process where embryos that can develop into whole plants are produced from somatic cells rather than through the fusion of gametes (Guan *et al*., 2016). SE in orchids can be divided into direct and indirect SE. Direct SE consists of the formation of embryos directly from isolated cells without the formation of callus tissue. Indirect SE is characterized by the formation of callus as a stage that precedes the formation of somatic embryos (Yelnititis, 2018). SE induction via somatic cells that can grow into bipolar plants possessing the same characteristics as their parents without gamete fusion produce superior seeds in bulk, this technique also aims to improve plant quality,

it can produce large numbers of plant seedlings in a short, can be used in germplasm or endangered genotype conservations (Mose *et al*., 2020).

Several factors influence the induction of somatic embryogenesis in orchids, including explant, the composition of culture media, and the application of plant growth regulators (PGRs) (Yelnititis, 2018). Among these factors, one crucial element is the use of PGRs. The combination of auxin and cytokinin is the most utilized PGRs during the initiation of SE in orchids (Shen *et al*., 2018). Auxin serves as a pivotal regulator in the early stages of SE establishment and post embryonic plant development, while cytokinins play a significant role in cell specification, differentiation, and dedifferentiation in plants (Asghar *et al*., 2023). Auxins like 2,4-dichlorophenoxyacetic acid (2,4-D), 1-naphthaleneacetic acid (NAA), and Indole-3-acetic acid (IAA) have been identified as effective inducers of SE in orchids (Juntada *et al*., 2015; Maulana *et al*., 2019; Sasmita *et al*., 2022).

2,4-D is the most frequently utilized PGRs due to its indispensable role in SE induction and early embryo development, and it exhibits optimal efficiency across various in-vitro cultures (Mahendran & Bai, 2016). 2,4-D is a synthetic auxin known for its resistance to enzymatic degradation and photooxidation (Budisantoso *et al*., 2017). Hany *et al*., (2023) reported that the concentration of 2 mg/L 2,4-D successfully induced direct SE in *Dendrobium discolor*. Astuti *et al*., (2019) also observed that a concentration of 2 mg/L 2,4-D was effective in the induction of SE in *Vanda sumatrana*. SE formation from various explants of *Epipactis veratrifolia* orchids use a combination of 2,4-D and cytokinins (Moradi *et al*., 2017).

Thidiazuron (TDZ) is one of the most widely used plant growth regulators for the induction of somatic embryogenesis. TDZ was found to be effective for the induction of SE in low concentrations (Parthibhan *et al*., 2018). TDZ has been demonstrated to be the most effective in SE induction compared to other purine-type cytokinins (Bhattacharyya *et al*., 2018). Research by Juntada *et al*. (2015) demonstrated that the combination of 0.1 mg/L TDZ + 1.0 mg/L 2,4-D was the most effective for SE induced in *Dendrobium sonia*. The highest percentage of SE was found in the treatment of 0.3 mg/L TDZ + 0.1 mg/L NAA in *Oncidium* sp (Shen *et al*., 2018). Benzyl-amino purine (BAP) is a type of cytokinin the most often used in cell differentiation because it is more stable, less expensive and more effective, and resistant to degradation (Rustikawati *et al*., 2021). The percentage of explants forming SE was highest for orchids on media containing low concentrations of BAP. The combination of 0.5 mg/L BAP + 0.5 mg/L NAA has been showed to induce SE in *Dendrobium ovatum* (Pyati, 2022). *Dendrobium lasianthera x Dendrobium antenna used* 0.025 mg/L BAP + 3.0 mg/L NAA, which was the most effective PGRs for SE induction (Sasmita *et al*., 2022).

Based on the explanation above, research had been done entitled "Effects of Auxins and Cytokinin Combinations on Somatic Embryo Induction of *Dendrobium mussauense* Ormerod". The research needed to be observed to complete data related to used of combination auxins and cytokins on somatic embrto induction in orchids. The method is expected to be used to propagation of *D. mussauense* for conservation efforts.

1.2. Problem Formulation

Based on the description above, the formulation of problems in this research were:

1. Is the auxin and cytokinin combinations able to induction SE of *D. mussauense*?
2. In which concentration of auxin and cytokinin combinations that has the best result to induction SE of *D. mussauense*?

1.3. Research Objective

The objectives of this research are as follows:

1. To evaluate the auxin and cytokinin combinations able to induction SE of *D. mussauense*.
2. To evaluate the best concentration on the auxin and cytokinin combinations for somatic embryo induction of *D. mussauense*.

1.4. Research Benefits

The research benefits provides a scientific information effect effects of auxin and cytokinins combinations for somatic embryo induction of *D. mussauense*. The expected results are to be the first step in *D. mussauense* conservation efforts.