I. INTRODUCTION

1.1. Background

Indonesia has a very high biodiversity, especially in plants. Currently, many plants are endemic in certain areas of Indonesia. Endemic plants are plants native to an area that can not be found in another place. It is recorded by the International Union for Conservation of Nature (IUCN) that Indonesia has 25,000 species of plants, of which 15,000 species are endemic, and 386 species are endangered and two are already extinct (Kusuma *et al*., 2008). One example of an endemic plant of West Sumatera from the Zingiberaceae family is *Curcuma sumatrana* Miq. (Ardiyani *et al*., 2011). The leaves of *C. sumatrana* are used to wrap durian meat, which is then fermented as a cooking ingredient called pekasam. In addition, the boiled water of the leaves can be utilized to cure skin problems such as itching (Ardiyani *et al*., 2011).

The existence of *C. sumatrana* in nature is limited to only a few areas such as Maninjau, Sianok, Lembah Anai, Kayu Tanam, and Ulu Gadut from Bukit Barisan (Ardiyani *et al*., 2011). Status *C. sumatrana* is vulnerable based on the IUCN Red List (Nurainas & Ardiyani, 2019). This species has a very small population size and very limited distribution in nature, thus the existence of *C. sumatrana* is increasingly threatened, so conservation and *in vitro* propagation efforts are needed.

Tissue culture technique is used to grow parts of the plant (cells, tissues or organs) called explants, aseptically on an artificial medium to produce plantlets (mini plants) (Mastuti, 2017). Cultivation techniques with *in vitro* culture are relatively faster, intensive and sustainable, and also have succeeded in multiplying the number

of ornamental and medicinal plants including species from the Zingiberaceae *i.e, Curcuma mangga* Valeton & Zijp, *C. zedoaria* Roxb., *Elettariopsis* sp., *Kaempferia galanga* L., and *Zingiber officinale* Roscoe (Ikeda & Tanabe, 1989; Hoesen & Poerba, 1992; Lestari & Sri, 2005; Triningsih *et al*., 2013; Yulizar *et al*., 2014; Aulia *et al*., 2020).

The common medium used in plant propagation through *in vitro culture* is the Murashige and Skoog (MS) basal medium (Nurfadilah, 2016). In general, MS medium is suitable for the species of Zingiberaceae (Shagufta, 2009; Ermayanti *et al*., 2010; Faridah *et al*., 2011; Wahengbam *et al*., 2015; Khumaida *et al*., 2019). Modifying macronutrients as one of the components in the basic medium of plant tissue culture needs to be done to obtain a suitable formula for plant growth (Iranbakhsh *et al*., 2011). Based on Pratama & Nilahayati (2018), the reduction in the composition of the MS half strength (½MS) to MS quarter strength (¼MS) medium is still able to support plant growth *in vitro* due to the presence of endogenous hormones in explants. These hormones support the availability of macro and micronutrients in a reduced composition medium, so they still affect plant growth.

The research conducted by Bhojwani & Razdan (1996) on *Dendrocalamus* showed that using ½MS medium produced more shoots than full MS medium. Meanwhile, in the same plant, the lower use of macro elements in ½MS medium proves that it is better for plant growth (Islam *et al*., 2003). The reduction of MS medium composition in blueberry plants showed increased shoot and root formation (Tetsumura *et al*., 2008). Marlina (2009) cultivated *Anthurium* using a modified ½MS medium, which was better at stimulating the formation of *Anthurium* callus. The MS

and ¼MS media were still good enough to grow explants of potatoes in terms of plant height, number of roots and shoots (Setiawati *et al*., 2018). Modifying macronutrients on MS medium (KH_2PO_4 and NH_4NO_3) significantly affected shoot height, number of leaves, and number of roots of *Tacca leontopetaloides*. The media with 170 $mg.L^{-1}$ KH_2PO_4 and 1650 $mg.L^{-1}$ NH_4NO_3 and 340 $mg.L^{-1}$ KH_2PO_4 and 825 $mg.L^{-1}$ NH_4NO_3 gave the optimal root growth at 2-8 weeks after culture (Rudiyanto *et al*., 2018).

Amino acids are the building blocks of proteins that have various functions in plants, including supporting, transporting other substances, coordinating the activities of organisms, cell response to a stimulus, moving, protecting against disease, and accelerating chemical reactions selectively (Rasullah *et al*., 2013). This protein is one of the elements found in the composition of the growing medium as a source for the fast healing of plants. Adding growth-promoting components to growth media, such as amino acids, has significantly affected tissue culture in many species (Asharo *et al*., 2013). Several amino acids often used in plant tissue culture are glutamine, glycine, arginine, and cysteine. Glutamine plays an essential role in cell dedifferentiation, proliferation, and maintaining the potency of embryo explant and is indispensable for amino acid biosynthesis (Winarto, 2011).

Winarto's research (2011) proved that glutamine significantly stimulated the growth of explant shoots. In this study, the addition of glutamine to *Anthurium andraeanum* at a concentration of 250 $mg.L^{-1}$ induced an anther growth potential of up to 48% with 21% anther regenerating and 1.3 anther per callus forming agent. According to Asharo *et al*. (2013), adding 100 $mg.L^{-1}$ glutamine to the axillary explants of *Saccharum officinarum* varieties NXI 1-3 with *in vitro* culture affected the

number of leaves, leaf length, and leaf width. In the study by Lavanya *et al*. (2012), it was shown that giving 50 $mg.L^{-1}$ glutamine to *Hildegardia populifolia* Schott & Endl. plants produced the highest average axillary and apical amounts.

Research on modifying of MS macronutrients on *in vitro* propagation of *C. sumatrana* is rarely reported. Furthermore, the addition of glutamine to obtain a suitable composition for the growth of *C. sumatrana* has never been done. Thus, research needs to be conducted to conserve *C. sumatrana* using an *in vitro* technique.

1.2. Formulation of Research Problems

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

1. What is the effect of macronutrient modification on MS medium in promoting the shoots growth of *C. sumatrana in vitro*?
2. What is the effect of the glutamine in promoting the shoots growth of *C. sumatrana in vitro*?
3. How is the interaction between macronutrient modified on MS medium and glutamine in promoting the shoots growth of *C. sumatrana in vitro*?

1.3. Research Objectives

The objectives of the research are:

1. To determine the effect of macronutrient modification on MS medium in promoting the shoots growth of *C. sumatrana in vitro*.
2. To determine the effect of the glutamine in promoting the shoots growth of *C. sumatrana in vitro*

3. To determine the interaction between macronutrient modified on MS medium and glutamine in promoting the shoots growth of *C. sumatrana in vitro*.

1.4. Research Benefits

Based on the research, it is expected:

1. The result of this research will contribute to the conservation efforts of *C. sumatrana*, thus the existence in nature can be maintained.
2. This research will fill the treasury of knowledge and is expected to be a reference in the propagation technique of *C. sumatrana in vitro*.