CHAPTER 1: INTRODUCTION

1.1. Problem Background

Dietary fiber is an edible plant cell component that is resistant to enzymatic digestion and is indigestible in human digestive system which makes it beneficial to gut health (Dhingra, Michael, Rajput, & Patil, 2012). It is a familiar term in food industry nowadays since it is added to a lot of bakery goods, drinks, and meat products in order to increase its health benefits. There are two types of dietary fiber based on its water solubility. The insoluble fiber cannot be fermented by the gut microbiota, while the soluble ones can be fermented by gut microbiota, which makes this type of dietary fiber commonly used as prebiotic agent (Dhingra et al., 2012).

Inulin is one of the most common examples of soluble fiber. It has been studied as a prebiotic dietary fiber since the 1990s, but it had just been considered by the FDA as dietary fiber in 2018 (United States Food and Drug Administration, 2018). Inulin will be fermented by *Bifidobacteria* and *Lactococcus* in the intestines into short chain fatty acids (SCFA) in which lactate and gases are also produced. This combination of fermentation products will indirectly increase the viability of intestinal flora, decrease pH of intestine, and produce bulking effect which promote healthy digestion system (Maroufi, Karimi, Mehdikhanlou, & De Loose, 2018; Shoaib et al., 2016; van Arkel, 2013). Aside from its prebiotic function, inulin can also be used as carbohydrate-based fat substitute which is used to increase viscosity, water-holding capacity, mouthfeel and texture as well as promote gel formation in food products (Öztürk, 2016). Due to its many functions, inulin has been extracted commercially and added to several types of food products such as yogurt, ice cream, and cheese.

The major source of commercial inulin is the chicory root (*Chicorium intybus*) but there are several common crops that also contain high amount of inulin such as Jerusalem artichoke, wheat, and onion (Shoaib et al., 2016). Biosynthesis of inulin in general uses sucrose as the precursor and the process

is catalyzed by two main enzymes: sucrose:sucrose 1-fructosyltransferase (1-SST) and fructan:fructan 1-fructosyltransferase (1-FFT). These enzymes have trans fructosylating activity in which they transfer one fructosyl molecule to a sucrose molecule resulting in a fructose polymer. 1-SST enzyme catalyzes the first reaction in which two sucrose molecules are converted into trisaccharide 1-kestose. 1-FFT enzyme then catalyzes the transfer of fructosyl from 1-kestose onto another 1-kestose, other fructans, or a sucrose molecule depending on the plant species (van Arkel, 2013; Van Laere & Van den Ende, 2002).

Indonesia has always imported inulin for its ice cream and other dairy products production. However, there are also some local plants that were found to contain high amount of inulin. Gembili tuber (*Dioscorea esculenta*) is a type of yams (*Dioscorea spp.)* that are commonly found in Indonesia and are known to contain high amount of inulin (2.8 – 14.77% of its dry weight) (Winarti, Harmayani, & Nurismanto, 2012). Among the other yam tuber species, Gembili is one of those with the highest inulin content (Winarti & Saputro, 2013). Ever since the discovery, Indonesian government has been supporting the studies about inulin from Gembili (Ridarineni, 2015). Inulin has been extracted from Gembili and tested for its prebiotic activity which showed similar prebiotic activity with commercial inulin from chicory roots (Zubaidah & Akhadiana, 2013). In order to obtain its own inulin, Indonesian government had encouraged farmers to plant Gembili tubers to be extracted for its inulin content (Ridarineni, 2015).

However, the demand for inulin will be increasing rapidly in the future. According to Inkwood Research (2017), the global market for inulin is projected to increase with a forecasted CAGR of 9.50% from 2018 to 2026. Asia-Pacific is believed to be the fastest-growing region for inulin market and Indonesia is one of the countries that demand high amount of inulin to expand its food and non-alcoholic beverages market for the next five years (Inkwood Research, 2017). Hence, inulin production in Indonesia should not only rely on traditional extraction method but also on biotechnology method.

1.2. Problem Formulation

Although Gembili tubers contain high amount of inulin, relying on the crops for inulin extraction is not going to meet the demand for inulin in Indonesia. The crop itself will only produce 5-20 tubers annually (Lebot, 2008). Moreover, it requires a lot of time as well as large area of land in order to provide enough inulin. Thus, a new approach using biotechnology should be done to produce inulin in a more effective way.

Since the information regarding its biosynthesis is available, many studies tried to manipulate the genes of inulin-producing enzymes in order to increase the production of inulin in a crop (Ávila-Fernández et al., 2007; A. Kawakami, Sato, & Yoshida, 2008; Maroufi et al., 2018; Nell, 2007; Shoorideh, Peighambari, Omidi, Naghavi, & Maroufi, 2018). The first reaction in inulin biosynthesis is catalyzed by 1-SST enzyme, thus overexpression of this particular enzyme had been extensively studied. Several studies had also showed that production of inulin can be manipulated by isolating 1-SST gene from inulin-accumulating crops and transforming it into other crops or organisms (Ávila-Fernández et al., 2007; A. Kawakami et al., 2008; Maroufi et al., 2018).

However, the studies on inulin-producing genes in Gembili tubers is very scarce. This is due to the unavailability of genomic data of the crop. To understand more about inulin production in Gembili, isolation and identification of inulin-producing genes, such as 1-SST gene, should be performed. Aside from adding up genetic data of Indonesia's biodiversity, the resulting 1-SST gene sequence can be used as the preliminary step of producing inulin using biotechnology approach in the future.

1.3. Research Objectives

This research aims to isolate and identify 1-SST gene in *Dioscorea esculenta*. The scope of this project are as follows:

1. Designing primers to isolate 1-SST gene from *D. esculenta*
2. Amplifying the gene by using genetic cloning

3. Sequencing of 1-SST gene from *D. esculenta*

The resulting gene sequence will be published to add up genetic information of Gembili tuber as one of Indonesia's biodiversity. The gene sequence can be used as the preliminary data for inulin production using genetic engineering in the future.