I. INTRODUCTION

I.1 Background

Drought is regarded as the most widespread natural catastrophe, affecting natural ecosystems as well as a wide range of human activities such as agriculture, water access, energy, tourism, and fundamental human wellbeing (Haile et al., 2020). Agriculture, on the other hand, is the human sector most affected by climate change since it is directly dependent on rainfall and evapotranspiration, and water shortages reduce agricultural productivity and quality (Parsons et al., 2019). The yield drop induced by a dry year is expected to be between 1-20% (Musolino et al., 2018). Despite a predicted doubling of agricultural water demand by 2050, climate change is expected to reduce freshwater availability by 50% (Gupta et al., 2020). Particularly concerning are the impacts of global climate change on agriculture in certain regions particularly vulnerable to drought intensification, such as southern Europe, Africa, and Asia (Meza et al., 2020).

Drought produces morphological, physiological, and biochemical changes in plants that are connected to abiotic stress (Seleiman et al., 2021). Plants have developed various mechanisms to cope with drought conditions, such as increased root water uptake, reduced water loss by closing stomata, and activation of hormonal responses (primarily mediated by abscisic acid [ABA]), resulting in the production of specific metabolites and increased antioxidant activity (Gupta et al., 2020). These pathways have been widely explored in wheat (Sallam et al., 2019).

The adoption and development of drought-tolerant cultivars have been the primary technique for preventing and countering the negative impacts of drought on crops (Kapoor et al., 2020; Rosero et al., 2020). Exogenous administration of growth regulators, osmoprotectants, and plant mineral nutrients has been attempted, but the response under real-world settings has been restricted and irregular (Marthandan et al., 2020; Seleiman et al., 2021). According to recent studies, the rhizosphere and the associated microbiome are the primary modulators of plant tolerance to drought (Zia et al., 2021). Thus, the key improvements in enhancing drought tolerance in the field are being made in the rhizosphere, such as the addition of beneficial microbes, hydrogels, nanoparticles, and seed priming (Seleiman et al., 2021; Zia et al., 2021).

Bacteria from the family Rhizobiaceae are symbiotic bacteria that convert atmospheric nitrogen into nitrogen that the host plants can use. They are Gram-negative, as are all Pseudomonadota. They are also aerobic in nature and have rod-shaped cells. Many Rhizobiaceae species are diazotrophs that can fix nitrogen and are symbiotic with plant roots (Ferguson, 2017). There are six genera in the Rhizobiaceae family, namely, *Rhizobium, Sinorhizobium, Mesorhizobium,* *Allorhizobium, Azorhizobium,* and *Bradyrhizobium* (Okazaki et al., 2004). These bacteria can be considered as one of the compositions of biofertilizer that promotes plant growth and productivity through symbiosis and is widely accepted as an alternative to chemical fertilizer. Rhizobacteria colonize plant roots effectively and promote plant growth through direct mechanisms, i.e., the production of various phytohormones that controls plant growth, such as IAA; P-solubilizing activity, N_2 fixation, and biological control activity, among others (Alemneh et al., 2020; Deshwal et al., 2011). Other than that, rhizobium could also indirectly promote plant growth; for example, rhizobium can produce catalase enzyme that can prevent oxidative damage from reactive oxygen species (ROS) to plants by destroying cellular hydrogen peroxide (H_2O_2) to produce water (H_2O) and oxygen (O_2) (Nandi et al., 2019).

One of the most well-known models of the symbiotic association between plants and rhizobia is the nodulation of legumes' roots by rhizobia. Host legumes use rhizobia's fixed ammonia as a nitrogen source, and the host legumes provides carbon for the metabolism of rhizobia. As a result, root nodule symbiosis is mutually beneficial and important in agriculture as it helps accomodate the nitrogen cycle.

Figure 1. Example of root nodules (some are marked by red circles) attached to plant roots.

The formation of root nodules begins with an exchange of signals between the host plant and its microsymbiont, in this case, rhizobium (Oldroyd et al., 2011). The exchange, however, cannot start when the bacteria and the host plant are incompatible (Wang et al., 2018). When the host recognizes suitable bacteria, it causes cortical cell divisions to generate root nodule primordia while also starting an infection process to transfer the bacteria into the nodule cells (Wang et al., 2018). The infection begins with the formation of plant-made infection threads that begin in the root hair. Infection threads with dividing bacteria grow through the epidermal cell layer into the nodule cells, where they are released and absorbed in an endocytosis-like process (Ferguson, 2017). Individual bacteria in nodule cells are surrounded by a plant-derived membrane, producing an organelle-like structure termed the symbiosome, inside which the bacteria develop into nitrogen-fixing bacteroids (Wang et al., 2018). Lipochitooligosaccharide molecules excreted by bacteria, known as Nod factors, can facilitate this infection process (Debellé, 2013).

A good example of legume plants that are normally able to have a symbiosis with rhizobia is called legume cover crop (LCC). LCCs are well known for their ability to improve or rather reinvigorate soil in areas that were previously used for mining activities or areas that are relatively low in fertility and have high temperatures (Nursanti & Supriyanto, 2022). LCCs are normally planted purposely by farmers to improve the condition of the soil. One of the more popular LCCs in Indonesia is the *Pueraria javanica* (PJ) which is a subtaxon of *Neustanthus phaseoloides* (Ma'ruf & Zulia, 2017). PJ is popular in Indonesia because it has a great ability to improve soil quality in dried soil, arid condition, and even under fluctuating sunlight availability (Ma'ruf & Zulia, 2017). Those abilities that PJ possesses, are mainly due to its ability to have a symbiosis with rhizobia (Selfandi et al., 2021).

The PJ samples that were used in this experiment was taken from rubber tree plantation area located in east Kalimantan (specifically in the following coordinate; Latitude: 0.494021; Longitude: 117.213178). East Kalimantan is notoriously known for its hot climate as it is located on the equator. Temperatures may reach up to 34-36^{o}C. Much of the forest area in east Kalimantan has been reduced due to illegal logging and many mining activities; these have directly caused the soil to have an arid condition and prevented many areas from having a broad range of biodiversity (Edwin et al., 2023). The site or area from which the PJ samples was collected has the previously mentioned conditions; low biodiversity, arid condition, and relatively low soil fertility; although the area has those characteristics, the rubber tree plantation that has PJ as LCC thrived and grew normally as if the land itself is fertile, a contrast to the plants that grew nearby and did not have LCC. In addition, rubber tree plantations are normally a monoculture plantation, meaning that the plantation exists with the sole purpose of growing one or only a limited number of plant types (Liu et al., 2023). To achieve that, LCCs are normally planted in the vicinity of the targeted plant type, and it is also there to help the plantation defend against diseases or damages by other factors. So it would be interesting to see which type of rhizobia that exists on that plantation.

The symbiotic activity between rhizobium and plants increases soil fertility, improving crops' growth, and it may also reduce the production cost of purchasing fertilizer (Praveen & Singh, 2019). Understanding the diversity and characteristics of rhizobium strains that can be isolated from legume cover crop (LCC) and their ability to fix nitrogen could have significant implications for sustainable agriculture (Kebede, 2021). By finding out which type or strain of rhizobium that can thrive in different conditions and how effective is the symbiosis between such rhizobium and plants, we might be able to develop more effective biofertilizers and enhance plant growth and productivity while reducing the use of chemical fertilizers, thus reducing the costs of plant cultivation. Overall, this study will contribute to our knowledge of the role of rhizobia in LCC plant growth and productivity and may have important implications for sustainable agriculture.

I.2 Research Aim

This study aims to isolate and characterize rhizobium as nitrogen-fixing bacteria from *Pueraria javanica,* a legume cover crop (LCC) taken from the rubber tree plantation in Kalimantan that has suboptimal soil conditions (dry and cracked soil appearance, relatively low rainfall rate, and low plant diversity in the area). This study also aims to try to find rhizobium strains that can live in an unoptimal growing conditions (arid conditions) as a potential bacteria that can enhance bio-fertilizer quality.