I. INTRODUCTION

1.1 Background

About half of the world's population depends on rice as a food source. A staple crop throughout the world, rice provides up to 76% of Southeast Asians' caloric intake and more than 21% of human caloric needs. (Zhao *et al*., 2020). After corn and wheat, rice is the third most produced cereal crop worldwide. In the most recent harvesting year, more than 530 million metric tons of milled rice were produced globally. Asiatic nations have historically produced the most rice worldwide (Shahbandeh, 2025). The demand for rice is expected to rise; accordingly, for instance, worldwide consumption reached 520.4 million metric tons in 2022-2023, up from 437.18 million in 2008-2009. However, production for 2023-2024 is estimated at only 513.54 million metric tons, indicating a pressing need to enhance rice yields to meet future demands and ensure food security while preserving cultural practices and economic stability.

Rice usually grown in an environment with large amount of water and categorized as semi aquatic species (Muthayya *et al*., 2014; Ahmed *et al*., 2022). Rice requires a significant amount of water throughout its growing season, and losses due to drought can significantly impact production levels (Birthal *et al*., 2015). Mainland Southeast Asia is a key rainfed agricultural region facing growing vulnerability to droughts, yet the monitoring and analysis of agricultural and vegetative of droughts stress here is still insufficiently explored (Ha *et al*., 2023). Rice as important global crops naturally don't have the ability to cope with drought (Zafar *et al*.,2022). Drought happens when extended periods of unusually dry weather led to significant issues, such as damage to crops (Khush, 2005; Orimoloye *et al*., 2022)

Developing drought-resistant rice varieties is crucial to meeting increasing production demands during climate change. In 2003, a collaboration involving the Malaysian Nuclear Agency, the Malaysian Agriculture Research Development, and Universiti Putra Malaysia led to the creation of two mutant lines, MR219-4 and MR219-9, which thrive in low-water conditions. Further, gamma irradiation of MR219 produced NMR152 and NMR151, both of varieties demonstrate tolerance to drought and submergence through careful selection processes (Abdullah *et al*., 2010; Ahmad *et al*., 2023).

Rice is very susceptible to drought on their germination and early stage of growth. The lack of water on the germination stage and early stage of growth would affect anatomical, physiological, morphological, and biochemical properties of rice. The changes in those properties may lead to more severe problems with rice cultivation and decrease yield production (Panda *et al*., 2021). Although the early stage and germination stage of rice plays a crucial role on rice cultivation, the research investigating plant responses, especially the physiological responses of rice during drought stress on early stage of growth is still limited.

The study about responses of NMR151, NMR152 and several varieties of Malaysian indica rice during drought stress was conducted by Sujiman (2019) using Polyethylene Glycol (PEG) 8000. The experiment evaluated only rice varieties with higher than 80% germination rate after 7 days of germinating. The treatments of PEG 8000 vary from 0-10% resulting in NMR151 and NMR152 as tolerant on drought stress. Further study on responses of NMR151 to extreme drought stress in early stage of growth, coleoptile development, is needed to provide more information on this new Malaysian indica rice variety responses to drought.

The experiment about assessing rice drought tolerance in PEG-induced extreme drought was done in several research. Diana *et al*., (2017) used Indonesian local rice variety as their sample and resulted that some Indonesian varieties can survive under extreme drought up to 30% of PEG concentration. However, this study focuses on observing the rice during their reproductive phase and the quantity of the crop after being harvested. Mishra & Panda (2017) used PEG up to 36% in their experiment resulting in higher relative ratios for different parameters (root length and shoot length). Susilawati *et al.,* (2022) used high PEG concentration up to 25% to analyse root growth of rice during drought stress. Another study that uses PEG up to 25% was experiment from Hartyanto *et al.,* (2024) resulting in decrease of germination percentage. For comparison, Mustamu *et al.,* (2023) using PEG up to 50% to analyse response of drought stress in maize. The result shown decrease in some traits, *e.g.*, Shoot length, germination percentage, and root length along with increasing of PEG concentration.

Numerous studies about drought stress on plants now recognized that drought tolerance is controlled by various genes, including transcription factors, which help plants survive to environmentally challenging conditions. These genes hold enormous potential as valuable targets for improving crop breeding (Joshi *et al*., 2016). During signal transduction, transcription factors function as molecular switches that directly control the expression of target genes. In recent decades, a lot of research has been focused on identifying and studying various transcription factors that manage plants respond to drought conditions (Franco-Zorilla *et al*., 2014; Hrmova & Hussain, 2021)

One of the transcription factors that is important in plant response to drought conditions is *DEHYDRATION RESPONSIVE ELEMENT BINDING* (*DREB*). *DREB* proteins *C-REPEAT BINDING FACTOR* (*CBF1*), *DEHYDRATION RESPONSIVE ELEMENT BINDING 1a* (*DREB1A*), and *DEHYDRATION RESPONSIVE ELEMENT BINDING 2a* (*DREB2a*) have been discovered to recognize dehydration responsive element core sequence. From various study about over-expression of *DREB* genes, it is reported that over-expression of *DREB* genes has improved survival rates under severe drought and salt conditions on rice (Cui *et al*., 2011; Yoshida & Yamaguchi-Shinozaki, 2021)

Based on explanation above, this study focusing on morpho-physiological responses of two Malaysian indica rice varieties, *i.e.*, NMR151 and its parental MR219, to extreme drought condition at the early stage of coleoptile development. In this study, PEG 60000 is used for concentration up to 30% to create extreme drought stress under *in vitro* conditions. Furthermore, this study is also aimed at monitoring the expression pattern of *OsDREB2A* under PEG-induced extreme drought.

1.2 Research Questions

Based on the background of this study, the formulation of research problems is:

1. How do the morpho-physiological responses of coleoptiles in the Malaysian indica rice variety, NMR151 and its parental line MR219, differ under PEG-induced extreme drought?

2. At which PEG concentration is the growth of coleoptiles in the Malaysian indica rice variety, NMR151 and its parental line MR219, have approximately similar to that under the control treatment?
3. How does the expression pattern of *OsDREB2A* differ in coleoptiles of Malaysian indica rice variety, NMR151 and its parental line MR219, under PEG-induced extreme drought?

1.3 Research Objectives

Based on research formulation, the objectives of this study are:

1. To evaluate the morpho-physiological responses of coleoptiles in Malaysian indica rice variety, NMR151 and its parental line MR219, under PEG-induced extreme drought.
2. To determine the optimal PEG concentration that carry-out the growth of coleoptiles in Malaysian indica rice variety, NMR151 and its parental line MR219, have approximately similar to the response of control treatment under PEG-induced extreme drought.
3. To analyze the expression pattern of *OsDREB2A* in coleoptiles of Malaysian indica rice variety, NMR151 and its parental line MR219, under PEG-induced extreme drought.

1.4 Research Benefit

This research will provide essential insights into the physiological effects of extreme drought stress on NMR151 and its parental MR219, specifically during its early coleoptile growth and development. Understanding how drought influences these critical developmental phases will allow for more comprehensive understanding of rice resilience to environmental stress. The findings of this study could contribute to understanding the drought-tolerant mechanism in the NMR151 which originated from MR219, known as drought susceptible variety. The information gained from this study could be useful for agricultural practices to help mitigate the adverse effects of drought conditions, leading to more sustainable rice products in the future.