CHAPTER 3

SOMATIC EMBRYOGENESIS AND CALLUS INDUCTION IN RICE

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3.1 INTRODUCTION

Rice has been known as an important crop and as staple crops in the half world's population. As per the data provided by the United States Department of Agriculture (USDA), China's rice production amounted to 148.30 million tonnes (Mt), whereas Malaysia's production reached 1.83 Mt in 2021. Although the total amount of rice production seems to be high, rice cultivation activity is vulnerable to climate changes (Fukagawa & Ziska, 2019). About 35% or rice consumption in Malaysia was imported from neighboring countries such as Thailand, India, Vietnam, and Pakistan. Following the COVID-19 pandemic, Thailand made the decision to halt its rice exports to Malaysia. This move has placed Malaysia's rice supply in a precarious situation, contingent upon achieving a 100% self-sufficiency level (SSL).

Research conducted by Dorairaj and Govender (2023), has uncovered a multitude of challenges plaguing Malaysia's rice production system. These challenges encompass adverse weather conditions, suboptimal soil fertility and nutrient management, limited awareness and knowledge among farmers, apprehension towards genetically modified planting materials, and inadequate utilisation of technology.

In contrast, most developed country that plant rice, they used precision breeding with genetic editing for producing superior genotypes that are resistant to stresses and have a high yield capacity. To achieve this, precision breeding that involving the genetic modification are play a vital role for superior rice production (Mohamed et al., 2021). These require an efficient regeneration system in tissue culture technology using somatic embryogenesis (SE) or organogenesis.

Implementation of SE via efficient callus formation would lead the successful regeneration system that must be developed first; therefore, further studies in searching the critical parameters for successful protocols are on the way. SE is a powerful biotechnological tool that involves cells dedifferentiation, and subsequent regenerations means for propagation of elite genotype. Due to plasticity features of plant cells, they can undergo differentiation, dedifferentiated, and re-differentiated multiple times and later regenerate into whole plant. The impediment of SE exhibits a response that varies significantly depending on the genotype, necessitating overcoming this obstacle.

Therefore, it is imperative to optimise parameters associated with SE for the sustainable production of rice in the future. These will motivate researchers to embark, and study in detail factors related to SE that would enhance their potential. In this review, the key factors in SE are explored thoroughly, particularly for callusing of MR303 rice, a high-yielding Malaysian cultivar derived from MR256 and MR253 (Sunian et al., 2019). Since the cultivar can be grown in semi-fertile paddy fields and less susceptible to the harsh environment, the yield believed to produce up to 10 tonnes per hectare (t/ha).

3.2 EVALUATION OF CALLUS INDUCTION FACTORS

Callus cells have been defined as undifferentiated cells that are like meristematic cells with distinctive features such as small vacuoles and lack chloroplasts for photosynthesis. In SE, callus cultures (undifferentiated somatic cell) can re-differentiate into entire plants, if maintained under appropriate growth media. Most rice callus cultures need dark growth conditions and usually grow at 25°C ± 2.

Rice callus can be distinguished by structure (compact or friable form and loose apart from colour (white to yellowish is embryogenic while brown is non-embryogenic). The plasticity of cell towards the callus tissue enables the whole plant to regenerate when media components are optimised. The synergy between these callus induction and SE offers a strategic entry point to harness these processes for accelerated crop improvement and development of genetically superior rice varieties. Most rice varieties responded differently *in vitro*; therefore, various tissue culture procedures existed due to different genotypes and explant developmental phases but more challenging for *indica* rice (Tripathy, 2021). Previous callus induction literature had shown that various factors (plant growth regulators, gelling agents, carbohydrates, amino acids as well as nanomaterials) influenced callus induction capabilities (Noor et al., 2022; Pravin et al., 2011; Rahman et al., 2021; Repalli et al., 2019).

The optimisation of medium composition normally starts with studying the concentration and type of plant growth regulators, organic supplements, followed by carbohydrates, solidifying agents and physical factors like desiccation treatment and incubation conditions (Binte Mostafiz & Wagiran, 2018). The understanding of these factors is crucial prior to the establishment of regeneration protocols and improvement of the SE efficiency.

3.2.1 Plant Growth Regulator

Most researchers started with optimisation study with appropriate plant growth regulator (PGR) type and concentration first before other factors were evaluated. Auxins such as 2,4-dichlorophenoxyacetic acid (2,4-D) has been the most popular plant growth regulator for rice callus induction study. Study by Noor et al. (2022), discovered that callus formation was increased up to 96% at 3 mg/L 2,4-D for the Himalayan indica rice variety SR4. The application of 2,4-D did increase callus percentage in cultivar