

UNIVERSITI TEKNOLOGI MARA

**GENETIC STABILITY OF
CRYOPRESERVED
MR 219 RICE VARIETY REVEALED
BY INTER-RETROTRANSPOSON
AMPLIFIED POLYMORPHISM (IRAP)**

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ABSTRACT

Cryopreservation is widely applied by researchers to preserve genetic materials of a sample under a very low temperature using liquid nitrogen. Rice is possible to be stored and preserved under a very low temperature with the presence of cryoprotectants as to maintain cellular processes of the cells after cryopreservation. Two cryopreservation protocols which are dehydration and encapsulation-dehydration were used in this study to preserve genetic materials of an indica variety of MR 219 rice. The sterilised seeds of the MR 219 rice variety were cultured on MSO for four weeks to get aseptic seedlings as source of stem explants. Stem explants were then cultured on MSO for two weeks. The stems (5 mm in length) were excised from 2-week-old plantlet derived from stem explants that were cultured on MSO medium. For dehydration method, stems were dehydrated for 219 minutes in a sterile Petri dish with 3.0 g silica gel to allow only 20 percent of water contained in the stems. On the other hand, for encapsulation, stem explants and sodium alginate solution were pipetted in together before they were released into 0.2 M calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) solution for hardening and bead formation. The optimum time for good polymerization to form is around 20 to 30 minutes. All beads were then dehydrated in sterile Petri dish with 3.0 gram of dry silica gel was added in, for 180 minutes. After 8 days, all dehydrated-cryopreserved stems were analysed by using Inter-Retrotransposon Amplified Polymorphism (IRAP) and regeneration testing. None of the stems capable to regenerate and no expected band appeared on gel electrophoresis for dehydration method. Even though no regeneration recorded for encapsulation, but two bands at the sizes of 300 bp and 140 bp were detected via IRAP analysis. These two detected bands were the expected bands for MR 219 indica rice variety. Encapsulation-dehydration method is more convincing to retain MR 219 rice genetic resources due to the presence of nutritive bead made from sodium alginate. *Tos17* was the targeted retrotransposon as they were induced abundantly in tissue culture system. Varieties and culture period are two major factors that can influence the distribution of *Tos17* retrotransposon in tissue culture. Based on the results obtained, it can be concluded that the best cryopreservation method for indica variety of MR 219 rice is encapsulation-dehydration.

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Malnutrition has been found as prevalent condition in the increase of in-hospital mortality as opposed to any diseases like obesity (Zapatero *et al.*, 2013). It is also associated with chronic obstructive pulmonary disease (COPD), which is projected to be the third cause of global death by the year 2020 (McGhan *et al.*, 2007). Malnutrition is the condition whereby the body of a malnourished person has a difficulty to grow and unable to resist disease (World Food Programme, 2015). In other words, the malnourished body is not being equipped with enough food. It is also likely for a person whom is equipped with enough food to become malnourished in particular when the supplied food does not have proper amount of micronutrients like minerals and vitamins.

Almost all developing countries, including Malaysia, having rice as their staple food. Many research works have been carried out to improve the nutrients content in rice. The main objective of rice improvement programs is to meet some specific nutrients target like iron (Fe), zinc (Zn) and vitamin A (Sharifa *et al.*, 2012). Based on all these research works, breeding programs and also the potency of rice itself, the micronutrient levels in rice can be elevated which indirectly can lower and overcome the malnutrition problem (Bouis and Hunt, 1999).

MR 219 is the commercial rice variety developed by the Malaysian Agricultural Research and Development Institute (MARDI) and has been commercialized as a long-grain variety. This variety has high amount of yield and is able to be produced more than 10 metric ton/hectare per annum (Alias, 2002). The other good characteristics of this variety include resistance to bacterial leaf blight and blast, a short maturation period which is 105 – 111 days, high grain weight; 28 – 30 mg, and contain 200 grains/panicle (Zuraida *et al.*, 2011).

For the time being, there is no report regarding to the extinction of rice, but there are many reports on the diseases attacked which influence the production of some rice varieties, for example Barrio rice. Nowadays, farmers in the highland of Barrio, Sarawak, are facing a rice disease called 'Tungro' which could reduce the production as much as 90 percent (Carolyn, 2013). In a nutshell, *Tungro* is known as "Penyakit Merah" by the local people in