UNIVERSITI TEKNOLOGI MARA

EVALUATION OF THE SIMPLE SEQUENCE REPEAT (SSR) GENOTYPING OF *Elaeis oleifera* GERMPLASM

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CONFIRMATION BY PANEL OF EXAMINERS

I certify that a panel of examiners has met on 8th February 2018 to conduct the final examination of Wan Nurhayati Wan Hanafi on her Doctor of Philosophy thesis entitled "Evaluation of the simple sequence repeat (SSR) genotyping of *Elaeis oleifera* germplasm" in accordance with Universiti Teknologi MARA Act 1976 (Akta 173). The Panel of Examiners recommends that the student be awarded the relevant degree. The panel of Examiners was as follows:

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ABSTRACT

Special attention has been given to the second species of oil palm, *Elaeis oleifera* as it possess several interesting agronomic traits such as slow growth, higher oil unsaturation and disease resistance. Studying the variability of *E. oleifera* germplasm is therefore very important as it serves as a tool to select source of genetic diversity for the oil palm conservation programme. The objectives of this study were 1) to identify the polymorphic *E. oleifera* gSSRs for *E. oleifera*, 2) to measure the information content of *E. oleifera* gSSRs, 3) to unravel the genetic diversity of *E. oleifera* germplasm, 4) to determine the genetic differentiation of *E. oleifera* germplasm and 5) to assess the genetic structure of *E. oleifera* germplasm. MPOB has developed a collection of simple sequence repeats (SSRs) from *E. oleifera* genome. Initially, optimization of PCR conditions for analysis of the oleifera samples using *E. oleifera* gSSRs was carried out. A total of 316 *E. oleifera* gSSRs were tested to evaluate their usefulness to assess the genetic diversity and population structure of *E. oleifera* populations. The PCR conditions were optimized while keeping the original DNA concentration, annealing temperature (T_A) and other reagent constant. Out of 316 *E. oleifera* gSSRs screened, 270 produced amplicons and of these numbers, 140 were polymorphic and potentially useful for diversity analysis. The modified PCR condition increases the success of amplifying *E. oleifera* gSSRs in the *E. oleifera* DNA samples analyzed. The PCR methods together with the polymorphic *E. oleifera* gSSRs were applied in genotyping the entire *E. oleifera* populations. A set of 21 polymorphic *E. oleifera* gSSRs was analyzed on a total of 214 *E. oleifera* genomic DNA belonging to eight germplasm originated from four countries in Central and South America (Columbia, Panama, Costa Rica and Honduras). The analysis covered on genetic diversity and genetic structure of eight *E. oleifera* populations, inferences from 21 polymorphic *E. oleifera* gSSRs. The average observed heterozygosity across population (H_o=0.249) was less than the expected heterozygosity (H_e=0.363). The highest population diversity was obtained in population 08 from Columbia (H_e=0.460±0.055, I=0.870±0.121. Eight of 21 polymorphic *E. oleifera* gSSRs were informative with PIC>0.5, where sMo00131 is the most informative (PIC=0.853). The populations analysed showed great genetic differentiation (F_{ST}=0.223). The Nei genetic distance showed the highest genetic distance was between population 01 from Columbia and population 02 from Costa Rica (0.555) while the lowest was between population 02 and population 03 from Honduras (0.019). The eigenvalues of PCoA plot showed that the first two components explained 38.70% of the total variation, which roughly ordinated the *E. oleifera* individuals into three major groups. Construction of neighbour-joining (NJ) tree separated *E. oleifera* individuals into two clusters. Model based clustering revealed that *E. oleifera* population has the highest ΔK when K was set to 7. The present study provides a diverse pattern of genetic diversity and the existence of genetic differentiation among *E. oleifera* germplasm. This study highlights the potential contribution of genetic variation of the *E. oleifera* collection analyzed using *E. oleifera* gSSRs for germplasm conservation and for utilization in breeding programs. Further conservation should focus on more populations with less number of palms per population development of core collection.

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CHAPTER ONE
INTRODUCTION

This chapter provides the background and rationale for the study. The overview describes the conservation of plant genetic resources, potential of the DNA molecular analysis, MPOB germplasm collection and the rationale of *Elaeis oleifera* germplasm evaluation.

1.1 BACKGROUND STUDY

Nations have been interested in conserving plant genetic resources for food and agriculture as they play important roles in the sustainability of human life on this earth. Conservation of plant genetic resources has been carried out to avoid loss of valuable genetic resources and to meet the demand for future food supply and food security. The resources are disappearing on an unprecedented scale due to deforestation, developmental activities, urbanization, changes in agricultural practices as well as modern agriculture and introduction of new and uniform varieties (Rao, 2004). Conservation of plant genetic resources has been carried out using two approaches namely *in situ* and *ex situ* conservation. *In situ* conservation involves maintaining genetic resources within their natural habitat whereas *ex situ* conservation involves conservation of plant genetic resources in germplasm collections and genebank. These two types of conservation are complimentary to each other's where the in situ conservation focus on habitat protection, while the ex situ conservation aims to preserve the genetic integrity of populations and individuals (Ibars and Estrelles, 2012).

The ability to identify diversity of plant genetic resources provides tools to capture maximum variation and minimize losses to the resources (Astley, 1992). The assessment of diversity within and among plants population can be carried out by means of (1) plant morphological characterization; (2) biochemical characterization and (3) DNA molecular analysis. However, morphological and biochemical characterization methods merit less attention than DNA molecular analysis due to their inability to detect low level variations (Govindaraj *et al.,* 2015). The DNA