1. INTRODUCTION

1.1 Introduction

The family Arecaceae contains many commercially significant crop plants, such as date palm, coconut palm and oil palm. Oil palm (*Elaeis guineensis* Jacq.) is a valuable economically important source of vegetable oil, the most traded vegetable oil in the international market, and is increasingly used in the food industry. It is an arborescent monocotyledon native to Africa and now widely cultivated across Southeast Asia. Malaysia, Indonesia and Thailand are the main producers in Asia. Among these countries, Malaysia is the largest producer of palm oil and exported 14.68 million tons of palm oil products worth RM 19.62 billion. In the world's supply, it takes second place only to soy bean. In 2002 it produced 11.90 million tons of crude palm oil. Based on data from the Food and Agricultural Organization (FAO), Thailand is respectively the world's fourth and third largest producer and exporter of palm oil. Consequently, palm oil production represents a significant and important part of the Thai economy. However, there is much room for improvement because the current average yield of palm oil in Thailand is only 2.8 tons per hectare compared to 3.6 tons per hectare in Malaysia. Within a relative short period the area planted with oil palm has increased tremendously from 15,000 hectares in 1980 to 199,000 hectares in 2000, a growth rate of 7.8% per annum (the 2nd in the world ranking) (Basiron, 2002; FAO, 2003).

Based on the fruit structure, oil palm is classified as *Dura* (thick shell; less mesocarp), *Pisifera* (Shell-less; embryo rarely formed) and the commercially cultivated *Tenara*, the D×P hybrid (thin shell; more mesocarp: 60-95%), with high oil content. There are, therefore, three important cultivars of oil palm, namely *Dura*, *Pisifera* and *Tenera*. Improvement in oil palm production, especially for oil yield, has generally been achieved via conventional crosses between *Dura* and *Pisifera* cultivars. *Tenera*, an F1 hybrid resulting from this cross, contains desirable traits from both parents. As oil palm is normally propagated by seeds, this results in a high variation in the field and available sustainable genetic trait production in traditional breeding is limited, the low palm oil yield is obtained. Not only is it difficult to propagate true-to-type plants by seeds because of the heterozygous nature of this species, but also no natural means of vegetative propagation is available for oil palm. Therefore, it is necessary to develop vegetative propagation to produce large number of plants from elite palm.

At present, plant micropropagation is applied for plant breeding in order to overcome some limitations of the conventional breeding, and clonal propagation of oil palm through tissue culture is common (Rabechault *et al.*, 1968; Aberlenc-Bertossi, 1991; de Touchet *et al.*, 1991; Teixeira *et al.*, 1995; Rajesh *et al.*, 2003). Plant regeneration was mostly achieved through callus or cell suspension cultures, and somatic embryogenesis seems to be satisfactory for oil palm propagation. One way for increasing the desirable genetic traits of oil palm for the highest quantity and quality of palm oil is by genetic engineering improvements, which can help to introduce required new characters from other organisms. The use of genetic transformation paves the way for the use of a wide range of beneficial specific genes.

Furthermore, this method allows the introduction of only desirable traits to pre-existing and desirable genotypes within a limited exhibition.

There are many genetic transformation techniques such as *Agrobacterium*–mediated transformation and micro-injection. Each method has a specific requirement which affects productivity. Although *Agrobacterium*–mediated transformation has several advantages such as no requirement for special equipment, techniques or protoplast culture systems, specific gene transfer to target tissues cannot be assured. For species that are not susceptible to infection by *Agrobacterium*, micro-injection by delivery of specific DNA into plant cells is currently the most useful technique. This technique is therefore expected to enable the transfer of desirable traits, such as quantity and quality of oil yield, for the new cultivar of oil palm. However, an efficient system of oil palm protoplast culture and specific technique are pre-requisites.

In principle, each protoplast can reform a new cell wall and later initiate cell division followed by callus formation. This has a chance for genetic transformation of oil palm by using protoplast. Therefore, oil palm protoplasts are ideal targets for transformation by a variety of means such as micro-injection or electroporation and protoplast fusion. Recently, protoplast-based biotechnology approaches have been used to complement conventional breeding of many crop species, and oil palm can be improved by somatic hybridization and genetic transformation. In this context, information on protoplast culture of oil palm, known to be difficult, is scanty. There are no previous reports of whole plant regeneration from oil palm protoplasts. Indeed, oil palm protoplasts have previously been isolated from cell cultures (Bass and Hughes, 1984) and polyembryogenic cultures (Sambanthamurthi *et al.*, 1996), but the

conditions for isolation and cell colony formation need to be investigated more extensively. Since there is no existing information on using actively-divided explants as protoplast sources of oil palm, this study focuses on the potential to obtain the best protoplast source.

It is generally known that plant cells, tissues and organs can be heterogeneous in size, shape and metabolic function. Within a population, there can be significant variations between individual cells in their ability to grow, their rate of growth and cell cycle activity (Hall and Yeoman, 1987). Moreover, genome size of *in vitro* cultured tissues can be altered through changes in the chromosome number. This alteration can be detected by measuring cell nuclei DNA content by means of flow cytometry. More than 25 years ago, flow cytometry has been applied to estimate nuclear DNA content in several plant species (Bennett and Leitch, 1995; Dolezel *et al.*, 1998). It offers a simple, rapid, accurate and convenient method for determination of ploidy levels of DNA assessment and analysis the cell cycle of large cell populations (Winkelmann *et al.*, 1998; Dolezel, 1991). The use of an internal reference standard gave poor reading and unreliable results in peak qualities, probably resulting from interference between the staining solution and nuclear DNA of the two species (Amsellem *et al.*, 2001). Additionally, the choice and correct use of reference standards is another criterion which has been largely neglected (Dolezel *et al.*, 1998).

A database of ploidy level and DNA content is important in any breeding program of new plant cultivars. DNA content of oil palm is rather variable, especially due to the high potential of conventional cross breeding among *Dura* and *Pisifera* cultivars in the first filial hybrid, *Tenera*, trait. Although DNA content of *Elaeis* cv. *Tenera* was reported previously (Rival *et al.*, 1997), flow cytometric studies of the nuclear DNA

content in their parents, *Elaeis* cv. *Dura* and *Pisifera*, have not been described to date. In higher plants, cell division is a cyclic process which consists of cellular events and nuclear events. Somatic cells having 2C DNA content are in the G0 (non-cycling) phase of the cell cycle. Cycling cells subsequently enter S phase, wherein the nuclear DNA content is doubled, and then enter the G2 phase. Analysis of the nuclear DNA content *via* flow cytometry provides an accurate estimate of the proportions of the cells within the various phases of the cell cycle (Dolezel, 1991; Sandoval *et al.*, 2003). The proportion of cells in the G2 phase of the cell cycle can indicate the potential of the explant cells to actively divide because the cells in this phase of the cell cycle have a doubling of chromosomes, and cell division readily occurs.

Imbibition is generally known to promote seedling germination. It is a pre-requisite to reactivate protein synthesis, transcription, duplication and mitosis by releasing the blockage of cells in the G1 phase (Deltour, 1985; Berlyn *et al.*, 1987), indicating the entry of cells into the synthetic phase of division and doubling of chromosomes (Bino *et al.*, 1992). Few data are available concerning the impact of imbibition on absolute DNA amount, although DNA plasticity has been shown to occur in relation to germination and environmental factors (Ceccarelli *et al.*, 1997). Flow cytometric study will therefore be setup on oil palm embryos that have been imbibed for different periods and cell cultures that have been cultured by the most suitable conditions in terms of number of cells in the G2 phase of the cell cycle.

Our attention was focused on the potential to obtain the best protoplast source using investigation of cell cycle activity determined by flow cytometry. Moreover, the present study reports a protocol for producing viable protoplasts and for cell colony formation from different sources of oil palm by establishing a simple and reliable

procedure for increasing of the mitotic activity of oil palm cells which affect protoplast cultures.

1.2 Objectives

1. To germinate oil palm seedling and induce callus formation.
2. To determine the proportion of cells in cell cycle in different sources of oil palm by flow cytometry.
3. To produce viable protoplasts and cell colony formation from different sources of oil palm.