

# PHYSICAL MAPPING OF rRNA GENES ON *Elaeis* CHROMOSOMES

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**P**hysical mapping of rRNA genes by in situ hybridization was performed on *Elaeis* species chromosomes. Fluorescence in situ hybridization (FISH) was performed on *Elaeis oleifera* chromosomes with probe pBG35 which contains 18S-25S rRNA genes from flax. Hybridization sites were located on the telomeric regions of an acrocentric pair of chromosomes and their satellite DNAs. Multiple labelling FISH was used to physically map the 5S and 18S-5.8S-25S rRNA multigene family in the interspecific hybrid of *E. oleifera* x *Elaeis guineensis* (OxG). Two heterologous labelled probes with 18S-25S (pTa 71) and 5S (pTa 794) rDNA were used for in situ hybridization of these chromosomes. It was found that 5S genes are situated on the proximal arms of the longest chromosome pair, or pair No. 1. The 18S-5.8S-25S rRNA genes are located on the telomeric regions of an acrocentric pair of chromosomes and on the satellite DNAs. The technique can now be used to locate other sequences of interest in the *Elaeis* species.

## Keywords:

In situ hybridization, rRNA, *Elaeis*, oil palm.

## INTRODUCTION

*E. guineensis* and *E. oleifera* (the two species of oil palm) belong to the monocotyledonous family *Palmae* which contains over 225 genera and 2600 species. The chromosome numbers of 111 genera and approximately 250 species, including *E. guineensis*, have been reported (Palomino and Quero, 1992), but little is known about their genome structure and organization. *E. guineensis* (*tenera*, DxP) palms have been grown commercially in Malaysia since the 1920s, and from the 1960s on, the palm oil industry began to prosper. *E. oleifera*, *E. guineensis* and their interspecific OxG hybrids have been utilized by oil palm breeders for their interesting traits. The *E. guineensis* is important commercially for its high oil yield, *E. oleifera* for high iodine value

(I.V.) oil and low height increment and finally interspecific OxG hybrids for high I.V. oil and low height increment.

The two *Elaeis* species in the genus contain  $2n=32$  chromosomes (Madon *et al.*, 1998) and the genome size of *E. guineensis* is  $1.8 \times 10^9$  base pairs (Jones *et al.*, 1982). However, Rival *et al.* (1997) reported the genome size as  $3.4 \times 10^9$  bp. The results differ due to the different methods and standards used. The former used microdensitometry while the latter used flow cytometry technique. At the chromosomal level, Madon *et al.* (1998) suggested that, based on length, *E. guineensis* and *E. oleifera* chromosomes can be divided into three groups of largely sub-metacentric chromosomes consisting of a large pair, No. 1 (10.89% and 11.05% respectively of the total length of the haploid complement, group I), eight medium size chromosomes (5.50%-8.90% and 6.28%-8.01% respectively of total length, group II) and seven small sub-telocentric chromosomes (3.62%-5.33% and 3.24%-5.72% respectively of total length, group III). The structure of the oil palm genome has been analysed cytologically and by molecular cytogenetic methods such as FISH. In this study, FISH

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of 5S and 18S-25S heterologous ribosomal DNA probes were used to locate these sequences on the chromosomes of *E. oleifera* and OxG hybrids. Using these probes, nucleolar organizer chromosomes can be identified and used as marker chromosomes.

FISH is an extremely useful addition to the armoury of the plant cytologist, permitting detailed information on the chromosomes in both mitotic and meiotic preparations (Schwarzacher *et al.*, 1992; King *et al.*, 1993; Miller *et al.*, 1994). This approach is particularly important in the physical mapping of 5S and 18S-5.8S-25S rRNA genes. The number and chromosomal locations of 5S and 18S-5.8S-25S rDNA are the most extensively studied genetic units in the plant genome (Linares *et al.*, 1996). Ribosomal genes possess three general characteristics: (i) they occur as tandemly repeated units with a copy number varying from hundreds to thousands per haploid genome, (ii) they are generally clustered at one or more chromosomal locations, and (iii) they represent two of the highly conserved families of repeated sequences in plants (Lapitan, 1992). As these sequences are highly conserved in nature, heterologous probes can be used to locate them on chromosomes. Many studies on *in situ* hybridization have been done using heterologous rRNA gene probes. Ricroch *et al.* (1992) used sequences from *Helianthus argophyllus* to locate these genes on the chromosomes of *Allium* species while Brown *et al.* (1992) located these sequences on white spruce chromosomes using the rDNA probe from soyabean. Maluszynska and Heslop-Harrison (1993), Leitch *et al.* (1993) and Schmidt *et al.* (1994) used ribosomal sequences from wheat to locate the genes on the chromosomes of *Brassica*, barley and *Beta vulgaris* respectively,

In most plant genomes, the 18S-5.8S-25S and the normally unlinked 5S rRNA genes are present as tandemly repeated units of genes and intergenic spacers at one or more sites. Changes in the rDNA repeat copy number at a single locus, and in the numbers and chromosomal positions of loci can be rapid (Castilho and Heslop-Harrison, 1995). Therefore, the study of rDNA copy number and site distribution is important for examination of species relationships and evolution (Mukai *et al.*, 1991; Maluszynska and Heslop-Harrison, 1993; Castilho and Heslop-Harrison, 1995; Galasso *et al.*, 1995). Actively expressed regions containing 18S and 25S sequences also show second-

ary constrictions and satellite structures at the nucleolar organizer regions (NOR) (Mukai *et al.*, 1991). The ribosomal loci of plant genomes can be easily visualized on chromosomes using *in situ* hybridization with heterologous ribosomal probes (Linares *et al.*, 1996; Fominaya *et al.*, 1997). This technique is useful particularly in the physical mapping of 5S ribosomal genes. In plants, C-banding, silver staining and ISH have been used in detecting 18S-5.8S-25S rDNA genes. However, only ISH has been capable of detecting 5S rRNA genes (Murata *et al.*, 1997; Schondelmaier *et al.*, 1997; Fominaya *et al.*, 1997; Prado *et al.*, 1996).

Ribosomal genes have been mapped in many species such as barley (Leitch and Heslop-Harrison, 1993), sugar beet (Schondelmaier *et al.*, 1997) and wheat (Cuadrado *et al.*, 1996). In *Elaeis* species and their hybrids, little is known about their genome structure, organization and NOR loci. With the advent of FISH technique and heterologous ribosomal DNA probes, cytological maps showing the locations of 5S and 18S-5.8S-25S rRNA genes can be obtained.

## MATERIALS AND METHODS

### Plant Material and Chromosome Preparation

Metaphase spreads of *E. oleifera* and the OxG hybrid were obtained by using enzyme digestion techniques modified from Schwarzacher *et al.* (1989). Briefly, root tips from a 20-year-old mulched *E. oleifera* (UP 1026) palm and 2-year-old mulched palms of the OxG hybrid (made available by United Plantations Berhad, Teluk Intan, Perak, Malaysia) were pretreated with 2 mM 8-hydroxyquinoline for 5-6 hr and then fixed in 3:1 absolute ethanol:glacial acetic acid. For each sample, 6-7 root tips were rinsed several times with enzyme buffer (0.01 M citric acid-sodium citrate, pH 4.6), and the terminal 1 mm from each root tip meristem was incubated in enzyme solution (2% cellulase and 20% pectinase in enzyme buffer) at 37°C for 1-3 hr. The softened tissues were then rinsed with enzyme buffer and placed on a glass slide with a drop of 45% acetic acid. A fine forcep was used to squeeze the root cap and expel the protoplast of meristematic cells. The protoplast suspension was then covered with a coverslip. A few layers of filter paper were placed over the coverslip and the suspension was squashed using firm thumb pressure. The edges of coverslips were sealed

with rubber solution to prevent drying. The slides were screened by phase contrast microscopy and slides containing several metaphase spreads with 32 well paced chromosomes were selected for hybridization. The slides were frozen with liquid nitrogen and the coverslips removed before dehydration in 70% ethanol (20 min), air drying and storage in a desiccator or -20°C freezer.

### Ribosomal DNA (rDNA) Probes

For FISH of the 18S-25S rDNA probe on *E. oleifera* chromosomes, the probe used was pBG35 (a gift from Dr T H Noel Ellis of the John Innes Centre, UK) which contains an 8.6 kb insert containing the rDNA of flax (*Linum usitatissimum*). This fragment contains 18S, 5.8S and 25S ribosomal RNA genes and intergenic spacers (Goldsbrough and Cullis, 1981). The probe was labelled with biotin-14-dATP by nick translation (Gibco BRL Bionick Labelling System) following the manufacturer's instructions. For double target *in situ* hybridization on the interspecific hybrid (*E. oleifera* x *E. guineensis*) chromosomes, the 5S and 18S-25S rDNA probes, pTa794 and pTa71, were used. The pTa71 is similar to pBG35 and contains a 9 kb *Eco*RI repeat unit of 18S-25S rDNA from the common wheat, *Triticum aestivum* (Gerlach and Bedbrook, 1979). The pTa794 contains a 410 bp *Bam*HI fragment of 5S rDNA from wheat (Gerlach and Dyer, 1980). Probe pTa71 was labelled with biotin-11-dUTP and pTa794 was labelled with digoxigenin-11-dUTP by random prime labelling.

### FISH Protocols

The technique developed was based on the methods of Heslop-Harrison (1991) and Leitch *et al.* (1993).

**18S-25S rDNA on *E. guineensis* and *E. oleifera* chromosomes.** The slides were pre-treated with 10 µg ml<sup>-1</sup> pepsin in 10 mM HCl for 10 min at 37°C, followed by incubation in 100 µg ml<sup>-1</sup> of RNase A in 2x SSC for 1 hr at 37°C and washing twice in 2x SSC. The chromosome preparations were stabilized in freshly prepared depolymerized 4% (w/v) paraformaldehyde in water for 10 min, dehydrated in a graded ethanol series and air dried.

The hybridization mixture, consisting of 50-150 ng µl<sup>-1</sup> of DNA probe, 50% (v/v)

formamide, 10% (w/v) dextran sulphate, 0.1% SDS and 300 ng µl<sup>-1</sup> of sheared salmon sperm DNA in 2x SSC, was incubated for 10 min at 70°C and chilled on ice. Then 30 µl of the hybridization mixture was added to the chromosome preparations and covered with a plastic coverslip. The hybridization mixture and the chromosomal DNA were denatured together at 80°C for 10 min, 55°C for 5 min, 50°C for 2 min, 45°C for 3 min and finally at 37°C overnight in a Hybaid Omnigene temperature cyclor to enable hybridization between the target and probe. The next day, the slides were washed in 2x SSC for 3 min at 42°C, twice for 5 min at 42°C in a 20% (v/v) formamide in 0.1x SSC, twice for 5 min at 42°C in 2x SSC, twice for 5 min at room temperature and finally twice for 5 min at room temperature in 4x SSC/Tween (0.2%). The washing steps will remove mismatched or unhybridized probe molecules before incubation in immunofluorescent reagents.

The slides were then blocked in 5% (w/v) BSA in 4x SSC/0.1% (v/v) Tween 20 for 5 min at room temperature. Then, the slides were incubated with 2 µg ml<sup>-1</sup> of FITC - avidin in detection buffer in a moist chamber at 37°C for 1 hr. Excess antibody was removed by washing the slides in detection buffer three times for 5 min each time at room temperature. This was followed by counterstaining with 2 ng ml<sup>-1</sup> PI (propidium iodide in Vectashield antifade media). The slides were then screened using a Carl Zeiss Axioplan epifluorescent microscope with suitable filters. Photomicrographs were taken using a MC 80 camera system on Kodak 1600 ASA colour negatives or Kodak Ektachrome 1600 ASA colour slides, scanned into and printed from Adobe Photoshop after contrast optimization of images only had been made.

**Multiple target labelling of 5S and 18S-25S rDNA on *OxG* hybrid.** The slides were pre-treated as in the above up to the detection with immunofluorescent reagents. For the detection of digoxigenin- or biotin-labelled probes, the slides were blocked in 5% (w/v) BSA in 4x SSC/0.1% (v/v) Tween 20 for 5 min at room temperature. Then, the slides were incubated with 2 µg ml<sup>-1</sup> of FITC anti-digoxigenin or 5-10 µg ml<sup>-1</sup> Cy3-conjugated avidin in detection buffer in a moist chamber at 37°C for 1 hr. Excess antibody was removed by washing the slides in detection buffer three times for 5 min each time at room temperature. This was followed by counterstaining with 2 µg µl<sup>-1</sup> DAPI (diamidino-

Little is known about the genome structure and organization of the NOR loci in *Elaeis* species and their hybrids. With the advent of FISH technique and heterologous ribosomal DNA probes, cytological maps showing the locations of 5S and 18S-5.8S-25S rRNA genes can be obtained. Furthermore, it is important to know the number of sites of major gene clusters within genomes in order to understand gene expression and evolution. Also, their physical locations can be studied relative to genes mapped by genetic methods and used as chromosome markers to investigate translocations and other aspects of genome evolution (Cuadrado *et al.*, 1995). These rDNA sites also help in the identification of individual chromosome pairs, particularly the NOR chromosomes. As reported by Stevenson *et al.* (1998), the combination of 18S-25S and 5S rDNA signals allowed the unambiguous identification of three chromosome pairs in the genomes of both *Allium cepa* and *Allium fistulosum*.

Even though FISH experiments involve costly items such as fluorescent stains, fluorescent conjugated compounds or nucleotides and the use of an epifluorescent microscope with suitable filters, the signal resolution produced is high due to the high contrast between signals and chromosomes. The slides have to be viewed quickly since fluorescence signals will degrade when exposed to light. Slides cannot, therefore, be kept for record. This method also enabled the reprobing or multiple labelling of two or more probes which have been labelled with different fluorochromes. From this study, it was observed that fluorescence *in situ* hybridization techniques are applicable to the chromosomes of the *Elaeis* species and these techniques will be used regularly to locate other sequences of interest due to the high signal resolution and its versatility.

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