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FLUORESCENCE *IN SITU* HYBRIDIZATION OF rRNA PROBE TO *Elaeis guineensis* (*Tenera*) CHROMOSOMES

KEYWORDS: Fluorescence *in situ* hybridization (FISH); *Elaeis guineensis* (tenera); chromosomes; nucleolar organizer regions (NORs)

MADON, M*, CLYDE, M M* AND CHEAH, S C*

* Palm Oil Research Institute of Malaysia, PO Box 10620, 50720, Kuala Lumpur, Malaysia

* Genetics Department, Faculty of Life Sciences, National University of Malaysia, 43600, Bandar Baru Bangi, Selangor Darul Ehsan, Malaysia.

Fluorescence in situ hybridization (FISH) was performed on Elaeis guineensis (tenera) chromosomes using the highly repetitive ribosomal ribonucleic acid (rRNA) gene probe, pBG 35, from flax. FISH of pBG 35 on interphase cells showed two hybridization sites, thereby indicating two nucleoli, while FISH on metaphase chromosome spread showed the presence of four nucleolar organizer regions (NORs).

INTRODUCTION

The *in situ* hybridization technique (ISH) was first used by Gall and Pardue in 1969 to detect the location of ribosomal deoxyribonucleic acid (DNA) in the oocyte nucleus of *Xenopus*, using a probe labelled with tritium. Since then, the technique has been improved by others. Pinkel *et al.* (1988) applied it to detect hybridization sites of trisomy 21 and translocation of chromosome 4 in human chromosomes by using fluorescent markers. The markers used included FITC (fluorescein isothiocyanate) (Fukui *et al.*, 1994), rhodamine (Thomas *et al.*, 1994) and DAPI (4',6-diamidino-2-phenylindole) (Leitch *et al.*, 1991). This technique is now known as fluorescence *in situ* hybridization, or FISH. FISH enables immediate and accurate visualization of hybridization sites. However, preparations have to be viewed quickly to avoid fading of signals: thus slides cannot be kept for record over a long period of time. This problem has been solved, however, with the advent of the CCD camera and image analysis system, where images can be stored and analysed (Fukui *et al.*, 1994).

The FISH technique has been used to detect single, low or multicopy genes on chromosomes (Appels and McIntyre, 1985; Hutchinson, 1983; Peacock *et al.*, 1981; Rayburn and Gill, 1985). In plants, this technique has been widely used to detect the highly repetitive rRNA sequences. Ribosomal RNA is encoded by tandemly

repeated DNA consisting of the 5.8S, 18S and 25S genes. These sequences are separated by non-transcribed regions or intergenic spacers (Choumane and Heizmann, 1988). The genes are located in the nucleolar organizer region (NOR). However, the 5S rDNA gene's location has been shown to be not at the NOR but elsewhere in the genome (Rogers and Bendich, 1987). 5S rRNA genes have been located by FISH in rice (Song *et al.*, 1993), barley (Leitch *et al.*, 1992) and sugar beet (Schmidt *et al.*, 1994).

The rRNA genes are a major multigene family and are organized in long tandem arrays in the genome. Their distribution and expression have been studied in detail in several plant species (Flavell, 1986; Rogers and Bendich, 1987). 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 chromosomes of *Allium* species while Brown *et al.* (1992) located these sequences on white spruce chromosomes using the rDNA probe from soybean. Maluszynska *et al.* (1993), Leitch *et al.* (1993) and Schmidt *et al.* (1994) used ribosomal sequences from wheat to locate genes on chromosomes of *Brassica*, barley and *Beta vulgaris* respectively.

Elaeis guineensis belongs to the family *Palmae*, which contains over 225 genera and 2600 species (Purseglove, 1975). However, the chromosome numbers of only 35 genera and 75 species including *Elaeis guineensis* are known (Imam, 1982). Technical difficulties in studying the cytogenetics of the *Palmae* family have impeded progress in this field. There appears to be not much information on the cytogenetics of oil palm. This is possibly due to the small size of the chromosomes and the difficulty in spreading them (Imam, 1982). The oil palm, *Elaeis guineensis*, contains 2n = 32 chromosomes. Madon (1995) and Madon *et al.* (1995) suggested that, based on length, oil palm chromosomes can be divided into group I (chromosome pair No. 1), group II (Nos. 2-9) and group III (Nos. 10–16). Their sizes range from 1.15 to 2.97 microns. The present study was carried out to develop a FISH technique for oil palm chromosomes using the highly conserved rRNA gene as probe. It is envisaged that localization of these landmark probes may help in future genome mapping efforts.

MATERIALS AND METHODS

Plant material and chromosome preparation

Root tips from germinated seedlings of *E. guineensis* (tenera) 3 to 4 weeks old were harvested and pretreated in 2 mM 8-hydroxyquinoline for 5–6 hours at 18°C and subsequently fixed in absolute ethanol-glacial acetic acid (3:1) at 4°C overnight. Chromosome spreads for FISH were prepared using a protoplast technique modified from the methods of Ambros *et al.* (1986) and Schwarzacher *et al.* (1989).

Ribosomal DNA (rDNA) probe

The probe used was pBG 35 (a gift from Dr T. H. Noel Ellis of the John Innes Centre, UK) which contains an 8.6 kb insert isolated from flax, *Linum usitatissimum* var. *Stormont cirrus* (Goldsbrough and Cullis, 1981). This fragment contains 5.8S, 18S and 25S ribosomal RNA subunits. Probe pBG35 was labelled with biotin-14-dATP by nick translation (Gibco BRL BioNick Labelling System) according to the manufacturer's instructions.

FISH using probe pBG 35

The FISH technique was developed and modified from the method of Pinkel *et al.* (1988). Slides were first incubated with RNase A (200 µg/ml in 2x Saline-Sodium Citrate, SSC + 1% Bovine Serum Albumin, BSA) for 1 hour at 37°C, washed twice in 2X SSC at room temperature for five minutes each time, dehydrated in 70% and 96% ethanol for five minutes each time at room temperature and then air dried.

The probe mix contained 8–10 ng/µl of biotinylated probe DNA, 40% (v/v) deionized formamide, 8% (w/v) dextran sulphate, 2x SSC,

20µg herring sperm DNA and 0.5% SDS. The probe mixture was denatured at 95°C–100°C for ten minutes and put on ice to prevent DNA from reannealing. Then 40µl of probe mixture was added on to a slide, and covered with a coverslip with the edges sealed with rubber solution. Slides were then denatured at 80°C-90°C for thirty minutes followed by hybridization in a humid chamber at 37°C overnight (at least 18 hours).

After hybridization, the slides were washed in 2x SSC at room temperature for five minutes, at 37°C for ten minutes and finally for five minutes at room temperature to get rid of any excess probe. Then the following steps were carried out:

(1) Detection with FITC-avidin.

After the washing steps, slides were incubated in 4x SSC/Tween 20 (1000 ml 4x SSC + 0.5 ml Tween 20) for five minutes at room temperature. This was followed by incubation in 200µl 4x SSC/Tween 20 + 5% BSA (0.2 g BSA + 4 ml 4x SSC/Tween 20) at room temperature for five minutes. Then 200µl FITC-avidin was added on to each slide (4µl 5mg/ml FITC-avidin + 996µl 4x SSC/Tween 20 + 5% BSA solution) and incubated at 37°C in a humid chamber for one hour. Slides were washed in 4x SSC/Tween 20 at room temperature for five minutes, before amplification with biotin anti avidin D.

(2) Amplification with biotin anti avidin D.

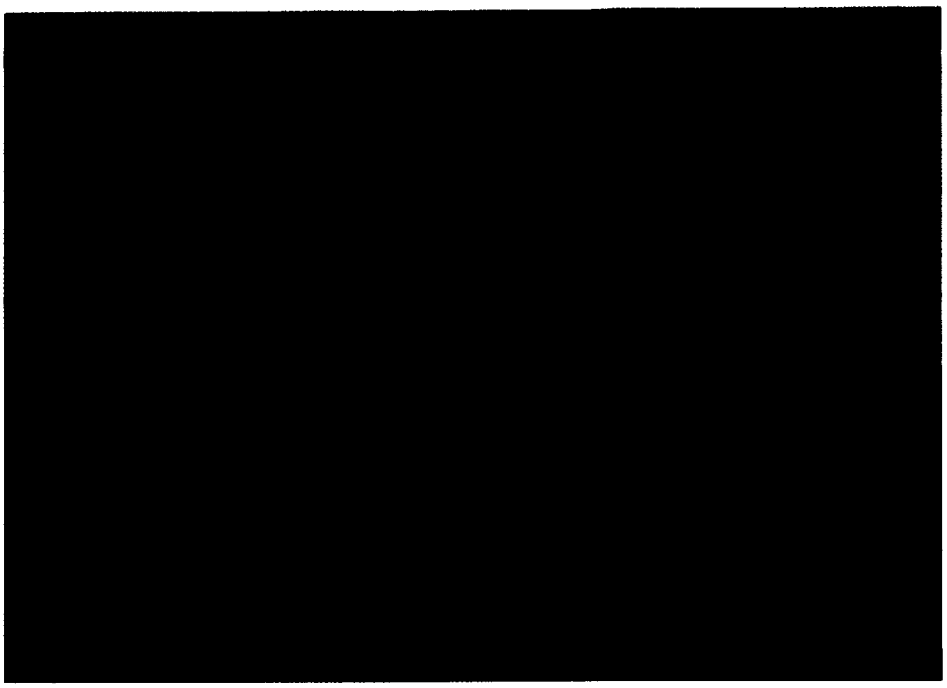
Two hundred µl of goat serum block (100µl of goat serum + 1900 µl 4x SSC/Tween 20) were added on to each slide, which was incubated for five minutes at room temperature. The goat serum block was discarded from the slide, and this was followed by incubation with 200 µl biotin anti avidin D (10 µg/ml) at 37°C for one hour in a humid chamber. Slides were washed in 4x SSC/Tween 20 at room temperature for five minutes. This step was followed by step (1) again.

After the detection step, slides were counterstained with propidium iodide (0.2µg/ml) for 45 seconds, then washed with tap water and blow-dried as quickly as possible. A drop of Vectashield (Vector Lab Inc.) antifade solution was placed on the slide and covered with a glass coverslip. Slides were pressed with a few layers of filter paper to absorb excess antifade medium and then the edges of the coverslip were cemented with nail polish to prevent drying of slides. Finally, slides were screened using a Zeiss Axioplan epifluorescent microscope with double band pass filter to allow simultaneous detection of FITC (which fluoresces yellowish green) and propidium iodide (fluoresces red). Photographs were taken using Kodak Ektar 1000 film. Microscopic visualizations were done after step (1) (detection with FITC-avidin) was performed.

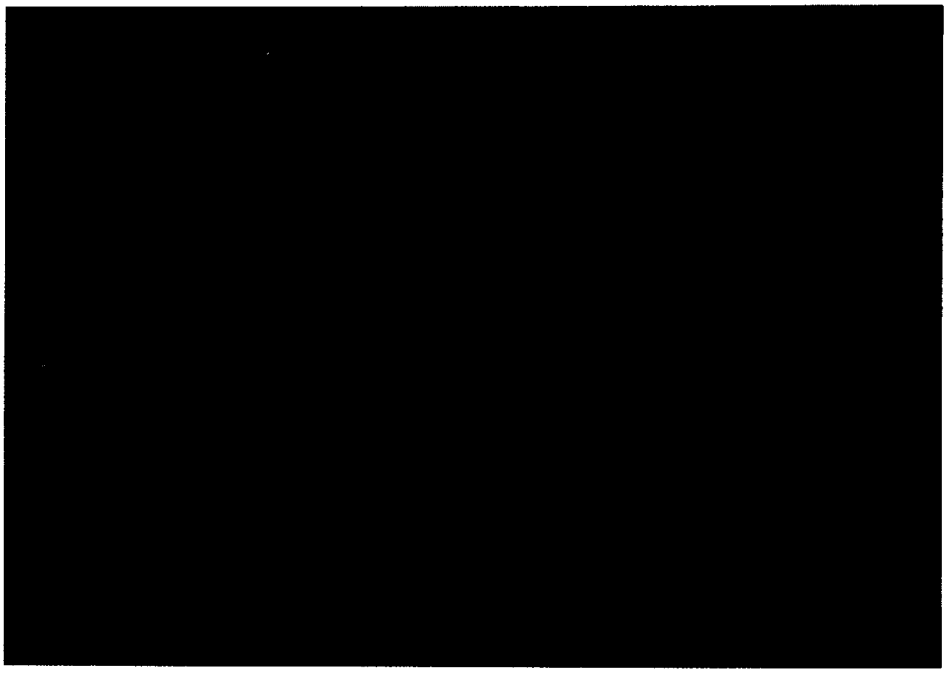
RESULTS AND DISCUSSION

Signals from microscopic visualizations were detected as fluorescing yellow dots on red chromosomes. *Figure 1* is a partial karyotype that shows two short chromosomes with signals on their telomeric regions. *Figure 2* shows the full set of chromosomes with four yellow dots indicating four hybridization sites. It is thus postulated that there are two pairs of nucleolar organizer chromosomes. Following step (2) (amplification with biotin anti avidin D), the preparations show enhancement of signals (*Figures 1b* and *2b*). More than ten interphase cells showed two hybridization sites, thereby indicating the presence of two nucleoli (*Figure 3*).

Figures 1a and *2a* show pictures taken after step (1) while *Figures 1b* and *2b* show pictures taken after step (1), (2) and (1) again. It was observed that the yellow dots in *Figures 1b* and *2b* fluoresced brighter than in *Figures 1a* and *2a*. Microscopic visualizations were carried out at the end of each step because the metaphase spreads had a tendency to be lifted off by subsequent washing. However, the areas surrounding the cells showed some fluorescence, probably because of FITC-avidin bound to the biotin molecules in the background. This may be caused by inadequate blocking of the bovine serum albumin and goat serum block. Background hybridization was observed as fluorescing greenish



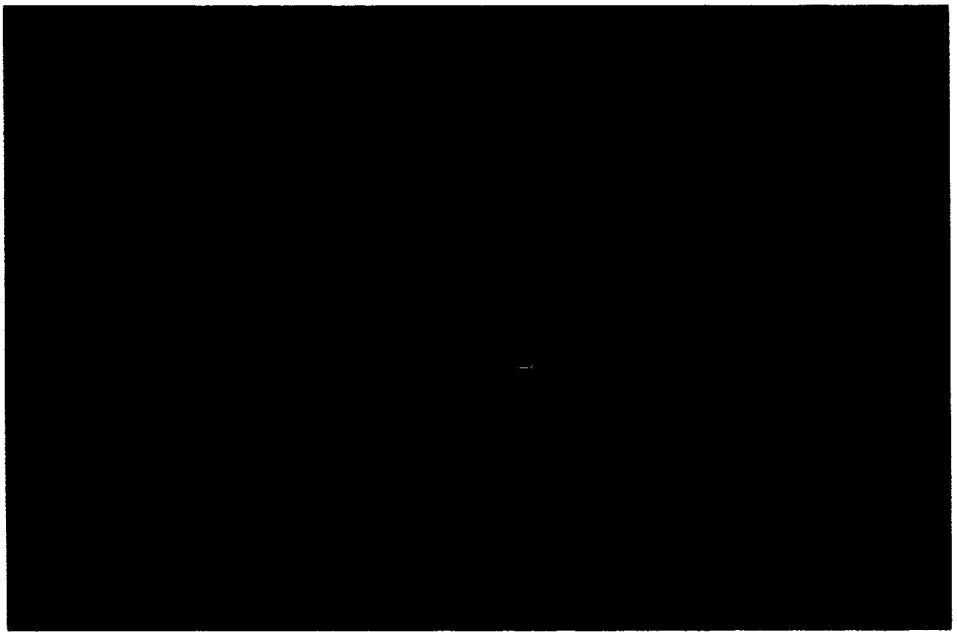
(a)



(b)

Figure 1. Two short chromosomes with signals on the telomeric regions: (a) prior to amplification, and (b) after amplification.

(a)



(b)

Figure 2. Full set of chromosomes showing four hybridization sites: (a) prior to amplification, and (b) after amplification.

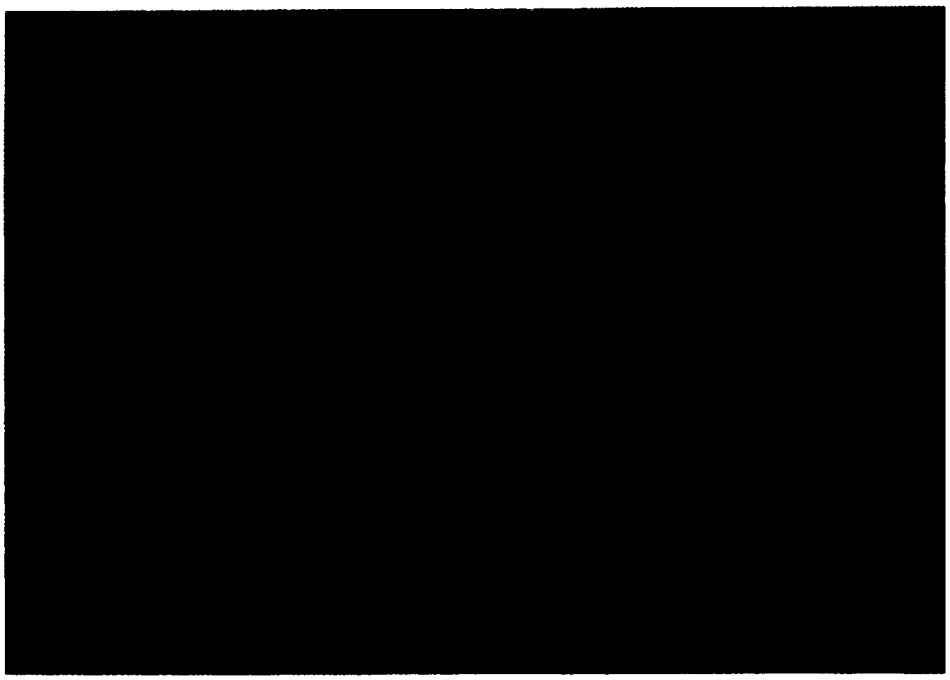


Figure 3. Interphase cells with two hybridization sites, thereby indicating the presence of two nucleoli.

compounds (*Figures 1b* and *2b*).

The work reported here is the first application of FISH to chromosomes of *E. guineensis* (tenera), an important crop species in Malaysia. We have demonstrated that the flax ribosomal gene probe hybridized with oil palm chromosomes, indicating that there is homology between the gene in these two plant species. Hybridizations of Southern blots containing oil palm DNA cut with restriction enzymes Bam HI , Eco RI and Eco RV with probe pBG 35 produced strong bands (unpublished data). This further indicates the homology between the ribosomal RNA genes of oil palm and flax. It can be concluded from this study that four chromosomes (two pairs) carry the nucleolar organizer regions (NOR) in oil palm. In support of this, it was observed that interphase cells showed the presence of two nucleoli. This observation is consistent with results of chromosome N-banding by Low (1990) who concluded that there are two pairs of nucleolar organizer chromosomes in the oil palm.

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ULTRASONIC STUDIES OF PALM OIL AND OTHER VEGETABLE OILS

Keywords: Ultrasonic properties; sound waves; attenuation; palm oil; palm olein

SIDEK, H A A ; CHOW, S P ; SHAARI, A H AND SENIN, H B*

* Ultrasonic Research Laboratory, Department of Physics, Universiti Pertanian Malaysia, 43400 UPM Serdang, Selangor, Malaysia

This paper reports the propagation and the attenuation of sound waves in crude palm oil (CPO), refined bleached deodorized (RBD) palm oil, palm olein and some other vegetable oils: coconut oil, corn oil and soyabean oil. The ultrasonic pulse echo overlapped technique has been employed to obtain the ultrasonic properties of the oils from room temperature up to 90°C. The velocity of sound in vegetable oil products decreases linearly with temperature, while their ultrasonic attenuation (α) decreases exponentially with temperature. By using an MBS 8040 ultrasonic analyzer, we observed that α/f^2 decreases nonlinearly with frequency (f), which is mainly due to a relaxation process. The ultrasonic properties of the oils are very dependent on their viscosity, density and molecular structure. The ultrasonic wave velocity and attenuation coefficient can be used as a basic tool to identify Malaysian palm oil.

INTRODUCTION

Ultrasonic techniques have been widely used to study a number of the physical properties of oils, *e.g.* in solid fat content determinations, the estimation of adiabatic compressibilities and the investigation of phase transitions (Bhattacharya and Deo, 1981; Hussein and Povey, 1984). The temperature dependence of the velocity of ultrasonic waves has also been employed to detect adulteration in a number of animal and vegetable oils (Rao *et al.*, 1980) and to determine particle size