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APPLICATION OF GENOMIC *In situ* HYBRIDIZATION (GISH) ON *Elaeis* HYBRIDS

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The introgressed parental genomes of Elaeis oleifera x Elaeis guineensis *(OxG) hybrids were investigated using the genomic* in situ *hybridization technique. This technique was able to show clear differentiation between the* E. oleifera *and* E. guineensis *genome. It was observed that there are* 16 E. oleifera *and 16* E. guineensis *chromosomes in the OxG hybrids. The interphase nuclei of the OxG hybrids had groups of chromosomes from both parental genomes in discrete, non-intermixed domains.*

INTRODUCTION

Elaeis guineensis and *Elaeis oleifera* are two species of oil palm. In Malaysia, only E. guineensis is planted commercially since it produces a high oil yield. *E. oleifera* produces a less saturated oil but has low oil yield. E. *oleifera* also has the advantage of slow height increment. To introgress the high unsaturated oil trait into E. guineensis, oil palm breeders have crossed the two species to produce OxG hybrids. The hybrids carry the advantages of the E. *oleifera* parent compared to E. guineensis: (i) the oil is more unsaturated, and (ii) the height increment is slower. However, the hybrids still have a poor oil yield. They are also vigorous in vegetative growth, making it difficult to harvest the fruit bunches. For these reasons, the OxG hybrid is backcrossed to its E. guineensis parent to improve the poor characteristics. Therefore, it would be useful to breeders to be able to assess the genomic composition of the backcross progenies and select only those with a high proportion of the guineensis parent.

The technique of genomic in *situ* hybridization (GISH) has been applied successfully on many species and it has been a valuable technique for identifying alien chromosomes and chromosome segments in wheat (Le *et al.*, *1989;* Mukai and Gill, 1991; Schwarzacher *et al.*, 1992;

Mukai et *al.,* 1993). For GISH to work, total genomic DNA from the introgressed alien species was used as probe, together with excess amounts of unlabelled blocking DNA from wheat. Ring et al. (1993; 1994) showed the technique to be a powerful tool in the study of chromosome pairing at meiosis in wheat/rye hybrids. Orgaard et al. (1995) have used this technique to investigate the parental origins of two Crocus cultivars, C!. 'Stellaris' (2n = 2x = 10) and C. 'Golden Yellow' (2n ,= 3x = 14), and found both to be hybrids of C. *flavus* (2n = 8) and C. *angustifolius* (2n = 12). Since GISH is fast, sensitive, accurate and informative, it could be developed to ascertain the amount of introgressed parental genomes into OxG hybrids.

MATERIALS AND METHODS

Plant Material, DNA and Root Tip Preparation

The F, individuals selected were Palms 66/23.91 (H66) and 80123.91 (H80) from the cross *E. oleifera* (UP10261 x *E. guineensis* (T128). The bases of the palms were mulched and root tips harvested from beneath the mulch. The materials were made available by United Plantations Berhad (Ulu Bernam and Teluk Intan, Perak). Root tips were cleaned and pre-treated with 2mM 8-hydroxyquinoline for 5-6 hours at 18°C. After pre-treatment, the roots were fixed in Carnoy's fixative (3: 1, absolute ethanol:glacial acetic acid) for 24 hours. Following a fresh change of Carnoy's fixative after 18 hours, the roots were finally stored in 70% ethanol at 4°C. Genomic DNA was isolated from the spear leaves of *E. oleifera* and *E.* guineensis using the cTAB method by Dellaporta et *al.* (1993). The DNA concentration was estimated using a spectrometer at A_{260} absorbance.

Chromosome Preparation

Chromosome preparations of OxG were obtained by using an enzyme digestion technique modified from Schwarzacher et al. (1989). Six or seven root tips were rinsed several times with enzyme buffer (0.01M citric acid-sodium citrate, pH 4.6) and an approximately lmm length cut from each tip meristem. This region can be identified by its opaque colour. These materials were incubated in an enzyme solution containing 2% cellulase (Onozuka R10, Yakult Honsha Co., Tokyo) and 20% pectinase (from *Aspergillus niger*, Sigma Chemical Co., St. Louis, Mo.) in citric-citrate buffer at 37°C for 2-3 hours. Softened tissues were rinsed in enzyme buffer and placed on a glass slide with a drop of 45% acetic acid. A pair of fine forceps was used to squeeze the root cap to expel protoplasts from the 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 squashed using firm thumb pressure. The edges of the coverslips were sealed with nail polish to prevent drying. The slides were screened by phase contrast microscopy and those containing several metaphase spreads with 32 well-spaced chromosomes selected for hybridization. The slides were frozen in liquid nitrogen and the coverslips removed before dehydration in 70% ethanol (20 minutes), air-drying and storage in a desiccator or -20°C freezer.

Preparation of Total Genomic Probe (TGP) and Block

The total genomic DNA of *E. oleifera* was labelled with biotin molecules using Bionick Labelling Kit (Gibco BRL). Blocking DNA of *E. guineensis* was prepared by autoclaving total genomic DNA for five minutes at 15psi to fragment the DNA into 100-300bp lengths. The amount of blocking DNA used was 90x more than the probe amount.

Genomic *In* situ Hybridization Protocol

The slides were pre-treated with 10µg ml^{-1} pepsin in 10mM HCl for 10min at 37°C, followed by incubation in 100µg ml-' of RNase A in 2x SSC for one hour at 37°C and washedtwicein 2x SSC. Chromosome preparations were stabilized in freshly prepared depolymerized 4% (w/v) paraformaldehyde in water for 10min dehydrated in a graded ethanol series and air dried.

Prior to the in situ. hybridization, the probe and block were mixed to a final concentration of 5µg ml^{-1} in a solution of 50% (v/v) formamide, 10% (w/v) dextran sulphate, 0.1% (w/v) SDS

(sodium dodecyl sulphate) and 2x SSC (Heslop-Harrison et al., 19911. This was followed by beating for 10min at 70°C and chilling in ice for five minutes. Then 40µl hybridization mixture were added to the chromosome preparations and covered with a plastic coverslip. The hybridization mixture and chromosomal DNA were denatured together at 80°C for 10min, 58°C for 30min and finally at 37°C overnight using a Hybaid Omnigene temperature cycler. This was to enable hybridization between the target DNA and probe. The next day, the slides were washed in 2x SSC for three minutes at 42°C, twice for five minutes at 42°C in a 20% (v/v) formamide in 0.1x SSC, twice for live minutes at 42°C in 2x SSC, twice for five minutes at room temperature and finally twice for five minutes at room temperature in 4x SSC/Tween (0.2%). The washing removed mismatched or unhybridized probe molecules before incubation in immunofluorescent reagents.

The slides were blocked in 5% (w/v) BSA in 4x SSC/0.1% (v/v) Tween 20 for five minutes at room temperature, and then incubated with 2µg/ml of FITC – avidin in detection buffer in a moist chamber at 37°C for one hour. Excess antibody was removed by washing the slides in detection buffer three times for five minutes each time at room temperature. This was followed by counterstaining with 2ng ml^{-1} 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 with Fuji or Kodak 1600 ASA colour film. The photomicrographs were scanned into and printed from Adobe Photoshop after contrast optimization of the images.

RESULTS AND DISCUSSION

In plant breeding and evolutionary studies, it is sometimes necessary to identify or discriminate between genomes of closely related genera or species and to identify parents in hybrid plants or ancestors of allopolyploid species. Therefore, the use of total genomic DNA (consisting of the entire DNA complement of a plant species) as a probe to chromosome spreads is a useful technique as it permits discrimination of the genomic origin of the chromosomes in hybrid plants and allotetraploid species. Also, it provides important information about nuclear organization and meiotic chromosome pairing configurations (Orgaard and Heslop-Harrison, 1994). Conventional methods give limited data on these.

The total genomic DNA of *E. oleifera* (used as probe) was able to show clear discrimination of the parental origin of the chromosomes in OxG hybrids (Figures *1* and 2). Unlabelled *E.* guineensis DNA used as a block increases the specificity of probing (Schwarzacher et al., 1989; Leitch et al., 1991; Anamthawat-Jonsson and Heslop-Harrison, 1992). The blocking DNA hybridizes to the sequences in common between the blocking DNA, probe DNA and chromosomal DNA in situ, thereby leaving mainly species specific sequences as sites for labelled probe hybridization (Orgaard and Heslop-Harrison, 19941. To discriminate more distantly related species, use of blocking DNA is often unnecessary (Schwarzacher et al., 1989, Leitch et al., 1991). For more closely related species sharing the same genome, e.g. *Hordeum vulgare* and *H. bulbosum*, or *Secale cereale* and S. *africanum*, blocking is essential. The same applies for *E. oleifera* and *E. guineensis.*

The genomic in situ hybridization technique developed is able to distinguish parental chromosome sets in both metaphase (Figures la and *lb)* and interphase (Figures *2a* and *2b*) preparations of OxG hybrids. In OxG hybrids, the genomic DNA from *E. oleifera* (labelled with biotin and detected with FITC) hybridized to 16 chromosomes of E. *oleifera* while the *E.* guineensis block DNA hybridized to 16 chromosomes of *E. guineensis.* In the interphase nuclei of OxG hybrids, it can be observed that the genomes from the two species occupied discrete and non-intermixed domains in the nuclei. It has previously been shown by three-dimensional reconstruction that the positions of chromosomes in squashed preparations reflect the true genome organization (Leitch et al., 19911. A similar separation of parental genomes in interspecific and intergeneric hybrids has been reported in plant species such as *Triticeae* (Bennett, 1982; Linde-Laursen and Jensen, 1991; Schwarzacher et al., 1992; Kosina

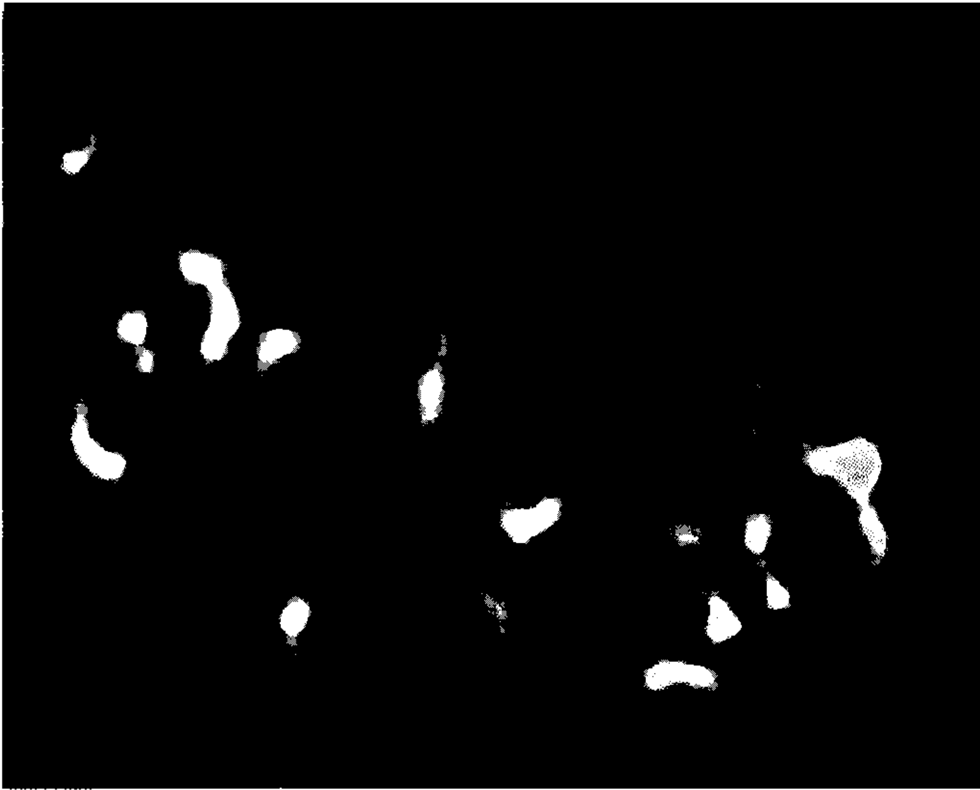
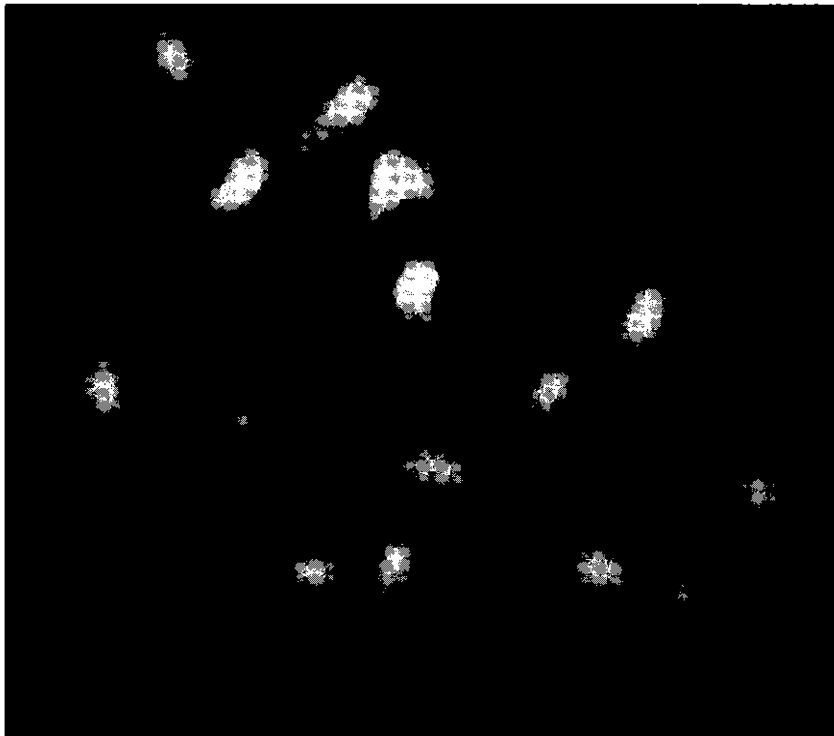


Figure 1a. Chromosome preparation from a root tip of OxG (H66) probed with biotin labelled total genomic DNA from E. oleifera *and blocked with genomic DNA from* E. guineensis. *Sixteen* E. oleifera *chromosomes fluoresced yellow while the* E. guineensis *chromosomes fluoresced red.*



re lb. Chromosome preparation from a root tip of OxG (H80) probed with biotin labelled total genomic DNAfrom E. oleifera *and blocked with genomic DNA from* E. guineensis. *Sixteen* E. oleifera *chromosomes esced yellow while the* E. guineensis *chromosomes fluoresced red.*

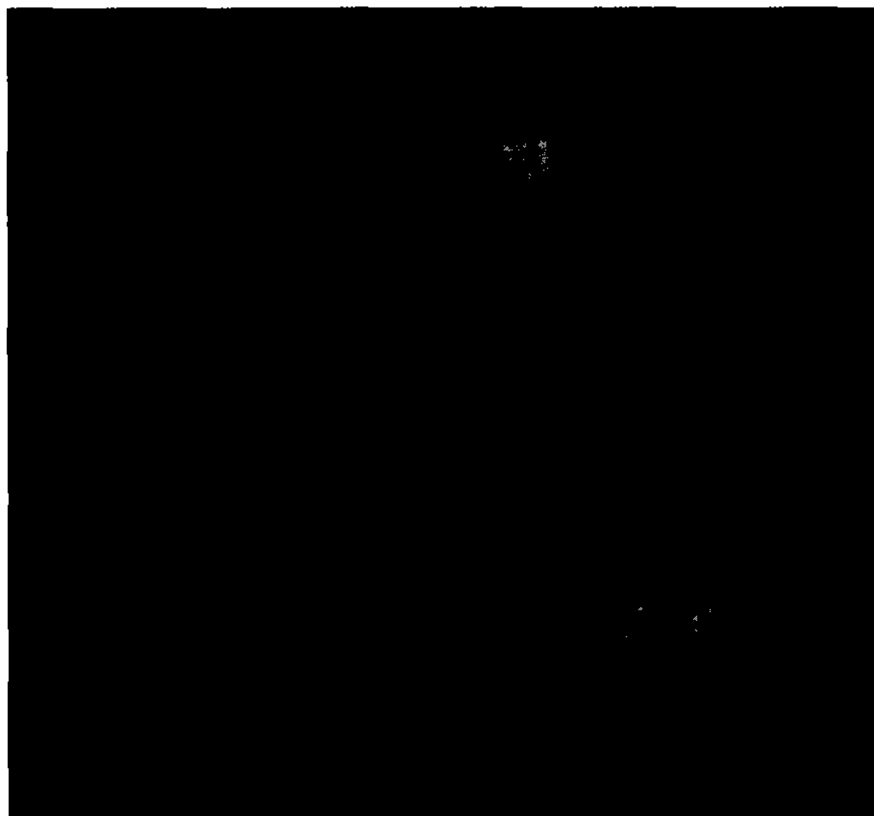


Figure 2a. At interphase, groups of chromosomes from both genomes occupy discrete, non-intermixed, domains in the nucleus of OxG (H66). E. oleifera genome *fluoresced yellow and* E. guineensis *genome fluoresced red.*

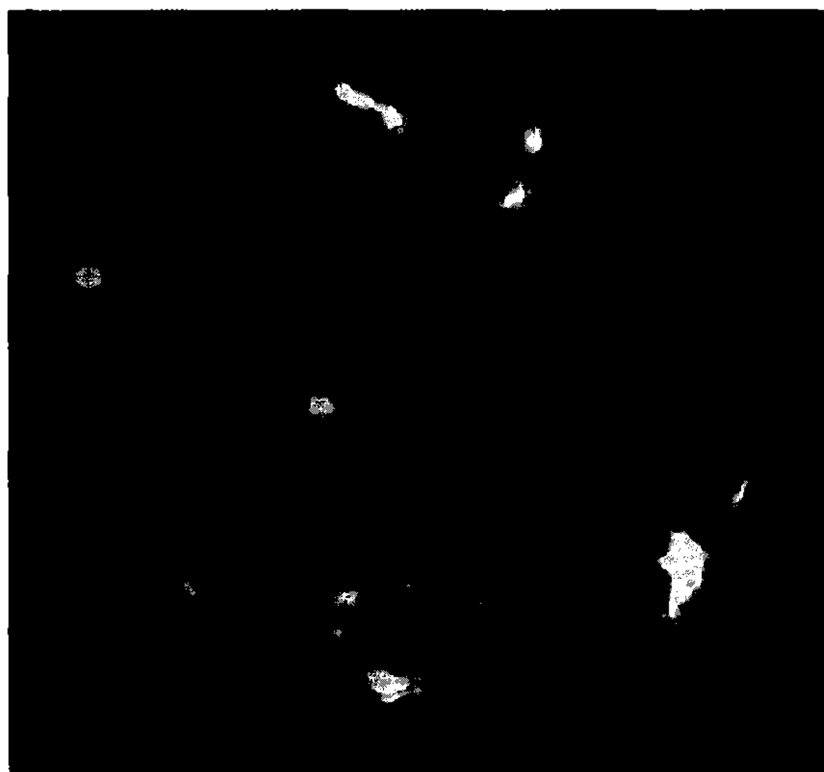


Figure 26. At interphase, groups of chromosomes from both genomes occupy discrete, non-intermixed, dom ains in the nucleus *of OxG (H80).* E. oleifera *genome fluoresced yellow and* E. guineensis genome *fluoresced red.*

and Heslop-Harrison, 19961, *Lathyrus* (Callimassia et al., 1994) and Crocus (Orgaard et al., 1995).

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