Cytogenetics Applications in Oil Palm Breeding: MPOB Experience

Maria Madon¹

ABSTRACT

Cytogenetics is a branch of genetics that is concerned with the study of the structure and function of the cell, especially the chromosomes. It is a useful application in plant breeding where integration of chromosome manipulation techniques and molecular cytogenetics (combination of molecular biology and cytogenetics disciplines) techniques with structural and functional genomics are essential to solve some of the plant breeding problems. This paper will describe MPOB’S experience in utilizing cytogenetics and molecular cytogenetics applications in oil palm studies and breeding aspects. Particularly in studying the chromosomal activities of both mitotic and meiotic cells, practical molecular cytogenetic applications such as fluorescence in situ hybridization (FISH) and genomic in situ hybridization (GISH) and finally on the oil palm pollen development for initiating efforts on microspore culture to produce haploid palms.

INTRODUCTION

The structure of the oil palm genome can be analysed by cytological and molecular cytogenetic means. Cytogenetics is a branch of genetics involved with the study of chromosomes and cell division. Fluorescent and non-fluorescent dyes can be used to visualize the chromosomes while utilizing simultaneously various banding and molecular cytogenetic techniques. For organisms to grow and reproduce, cells must divide. Mitosis and meiosis are both processes of cell division, but their outcomes are very different. The genetic purpose of mitosis (Figure 1) is to produce two daughter cells while meiosis (Figure 2) is to produce four daughter cells. Mitosis and meiosis occur in somatic and reproductive cells respectively. Figure 1 and 2 shows the schematic presentation and the stages of mitosis and meiosis. Cytological analysis performed on the meristematic root tip cells provide insights into the mitotic processes while meiotic processes can be observed in the reproductive organs such as anthers of male flowers.

¹ Advanced Biotechnology and Breeding Centre
Malaysian Palm Oil Board,
No 6, Persiaran Institusi,
Bandar Baru Bangi,
43650 Kajang
Selangor
Email: maria@mpob.gov.my, mariamadon@yahoo.com
Tel: 03-87694571, Mobile: 016-2151401
Molecular cytogenetics involves a combination of molecular biology and cytogenetics. In general, this involves the use of a series of techniques referred to as the fluorescence in situ hybridization (FISH), in which DNA probes are labeled with differently colored fluorescent tags to visualize one or more specific regions of the genome (Figure 6). The technology enables the location of DNA sequences such as ribosomal DNA, transgenes and specific ISSR and RFLP markers to identify individual chromosome pairs. Genomic in situ hybridization (GISH) uses one parent DNA genomic DNA as probe while the other parent DNA as block to distinguish the *Elaeis oleifera* and *E. guineensis* chromosomes in interspecific hybrids.

**METHODOLOGIES**

Plants are one component of the biosphere and the cell is a basic unit of structure in both plants and animals. In *Elaeis*, the meristematics cells of root tips and the pollen mother cells contained in anthers of the male flowers are used to perform cytogenetic analysis that involve mitosis and meiosis respectively.
Materials used to observe mitosis in oil palm

Figure 3. Shows (a) root tips and (b) callus tissues of oil palm

Materials used to observe meiosis in oil palm

Figure 4. Shows (a) oil palm male spikelets containing male flower buds, (b) a male flower bud and (c) the six pairs of anthers obtained from a male flower bud which contain the pollen mother cells
Flow chart of cytogenetic analysis

Table 1 showed the flow chart on studying the mitosis and meiosis in *Elaeis* or plants in general with slight modifications.

Table 1. Flow charts for cytogenetical analysis of mitotic and meiotic activities

<table>
<thead>
<tr>
<th>MITOSIS IN <em>Elaeis</em></th>
<th>MEIOSIS IN <em>Elaeis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Root tips or callus tissues</td>
<td>Male flower buds containing anthers</td>
</tr>
<tr>
<td>Squashing with stain ie. Aceto orcein (nonfluorescent) or DAPI-diamidinophenylindole (fluorescent)</td>
<td>Squashing with stain ie. Aceto orcein (nonfluorescent) or DAPI-diamidinophenylindole (fluorescent)</td>
</tr>
<tr>
<td>Microscopy visualization and image analysis</td>
<td>Microscopy visualization and image analysis</td>
</tr>
</tbody>
</table>
Flow chart of fluorescence or genomic in situ hybridization (FISH/GISH) experimental approaches

Figure 5. Fluorescence or genomic in situ hybridization (FISH or GISH) experimental approaches
Principle of FISH or GISH experiment to locate labeled sequences or probes

Figure 6. The principles of a FISH or GISH experiment to locate selected probes or labeled DNA sequences on chromosomes

APPLICATIONS (RESULTS)

1. Mitosis in meristematic cells (normal)

Cytogenetical analysis of meristematic cells able to illustrate whether the mitotic processes are normal or abnormal. Figure 7 show cells that undergo various mitotic activities without any chromosomal anomalies such as chromosome bridging (Figure 8a) and lagging (Figure 8b)
2. Mitosis in callus cells (abnormal)

Figure 8. Chromosomal anomalies such as chromosome (a) bridging and (b) lagging occurring in callus tissues

3. Karyograms of *Elaeis*

Using metaphase chromosome spreads and image analysis measurement software, karyotypes of plant species can be obtained. Figure 9 showed the karyotypes of *E. guineensis* and *E. oleifera*, the two species of oil palm. Both have 2n=32 chromosomes and the chromosomes are divided into Group 1 (pair No. 1), Group II (pair No. 2-9) and group III (pair No. 10-16).
Figure 9. Shows karyotypes of (a) *Elaeis guineensis* and (b) *E. oleifera*

4. **Meiosis in oil palm pollen mother cells (normal)**

The cytogenetic analysis performed on the anthers containing pollen mother cells (PMC) able to indicate whether meiosis occur normally or vice versa. Figure 10 show the normal progression of meiosis while Figure 11 shows examples of abnormal meiotic activities that can eventually lead to pollens with abnormal amount of DNA. The example in Figure 11 shows abnormal meiosis that occurs in PMC of a normal palm derived from tissue culture process.
Figure 10. Shows (a) Uninucleate microsporocyte or PMC (arrow) with two tapetum cells on the left, (b) Pachytene stage of microsporocyte chromosomes, (c) Chromosome bivalents condensing, (d) Further condensation of chromosome bivalents, (e) Diakinesis (Pairing of bivalent chromosomes), (f) Bivalent chromosomes at metaphase I stage of meiosis, (g) Chromosomes separating at anaphase I stage of meiosis, (h) Chromosomes at metaphase II stage of meiosis and (i) Tetrad stage of pollen mother cell or uninucleate microspore (arrow) prior to release from pollen mother sac.
5. Meiosis in oil palm pollen mother cells (abnormal)

Figure 11. Shows (a-d) chromosomes lagging (arrows) in a pollen mother cell (PMC), (e-f) abnormal divisions of nuclei as opposed to Figure 10i
6. Fluorescence in situ hybridization (FISH) experiments performed on oil palm metaphase chromosomes or interphase nuclei in the following applications:

(1) Locating DNA sequence of interest on the oil palm chromosomes.

Several examples follow.

a) 5S ribosomal DNA (Figure 12)

![Figure 12. Oil palm metaphase chromosomes showing 5S rDNA sites on the longest chromosome pair no. 1 (red arrows)](image)

b) 18S-25S ribosomal DNA (Figure 13)

![Figure 13. Oil palm metaphase chromosomes show 18S-25S rDNA sites located on the shortest acrocentric chromosome pair no. 16 (red arrows)](image)
c) Transgenes or plasmid pME22 carrying bar,phaC,bktB and phaB genes driven by maize polyubiquitine promoters for synthesizing PHB (Figure 14)

Figure 14. The transgene signals (red arrows) are located on the telomeric chromosomal regions

d) Specific ISSR and RFLP markers for oil palm chromosome identification
The chromosome number of E. guineensis and E. oleifera is 2n=32 (Madon et al., 1998), and can be divided into three groups on the basis of length. Group I consists of chromosome number 1 (longest), group II consists of chromosome numbers 2-9 (medium long) and group III consists of numbers 10-16 (medium short chromosomes). It is difficult to distinguish between the individual chromosome pairs, hence recently ISSR and RFLP markers that map onto the same linkage group have been used as probes in simultaneous double labeling FISH experiments. Table 2 shows the corresponding ISSR and RFLP markers used to distinguish between the individual chromosome pairs except for pairs numbers 3, 7, 11, 12 and 14. For chromosome pair number 16 the 18S-25S rDNA probe is used as its specific marker.
Table 2. The corresponding ISSR and RFLP markers are used to distinguish the following individual chromosome pairs except for chromosome pair number 16.

<table>
<thead>
<tr>
<th>Chromosome pair number</th>
<th>Specific RFLP and ISSR markers</th>
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<tbody>
<tr>
<td>1</td>
<td>RFLP markers: pOP-MET4, pOP-SFB56 and pOP-SFB82, and ISSR marker: UBC881-1500</td>
</tr>
<tr>
<td>2</td>
<td>RFLP marker : pOP-G246, and ISSR marker: UBC835/818-550/500</td>
</tr>
<tr>
<td>4</td>
<td>(a) RFLP marker: pOP-MT135, and ISSR marker: UBC836-780</td>
</tr>
<tr>
<td></td>
<td>(b) RFLP marker: pOP-MT194, and ISSR marker: UBC845-750</td>
</tr>
<tr>
<td>5</td>
<td>RFLP markers: M1C and M6B, and ISSR marker: UBC853-1050</td>
</tr>
<tr>
<td>6</td>
<td>RFLP markers: pOP-SFB154 and pOP-SFB147, and ISSR marker: UBC834-530</td>
</tr>
<tr>
<td>8</td>
<td>(a) RFLP marker: pOP SN1, and ISSR marker: UBC 825-750</td>
</tr>
<tr>
<td></td>
<td>(b) RFLP marker: pOP G16, and ISSR marker: UBC 890-725</td>
</tr>
<tr>
<td>9</td>
<td>RFLP markers: pOP-ME6 and pOP-ME51, and ISSR marker: UBC 823-715</td>
</tr>
<tr>
<td>10</td>
<td>(a) RFLP marker: pOP-SFB34, and ISSR marker: UBC 880/860-250</td>
</tr>
<tr>
<td></td>
<td>(b) RFLP markers: pOP-SFB34 and pOP G18, and ISSR marker: UBC 834-330</td>
</tr>
<tr>
<td>13</td>
<td>(a) RFLP marker: pOP-G39, and ISSR marker: UBC 880-980/900</td>
</tr>
<tr>
<td></td>
<td>(b) RFLP marker: pOP-MT30, and ISSR marker: UBC 830/834-300</td>
</tr>
<tr>
<td>15</td>
<td>RFLP markers: pOP-G39 and pOP-MT 40, and ISSR marker: UBC 808/834-700</td>
</tr>
<tr>
<td>16</td>
<td>pTA 71 (18S-25S rDNA)</td>
</tr>
</tbody>
</table>
• **Linkage group number 4 associated with Chromosome pair number 4**

With the following specific markers (Figure 15):

- **RFLP marker**: pOP-MT135
- **ISSR marker**: UBC836-780

Figure 15. Linkage group 4 (left) shows the locations of pOP-MT194 (RFLP marker, red oval) and UBC 845-750 (ISSR marker, green oval). Chromosome spreads (right) show hybridization of pOP-MT194 (red) and UBC 845-750 (green) on the same medium length chromosome pair.
Linkage group number 5 associated with Chromosome pair number 5

With the following specific markers (Figure 16):

RFLP markers: M1C and M6B
ISSR markers: UBC853-1050

Figure 16. Linkage group 5 (left) shows the locations of M1C and M6B (RFLP markers, red oval) and UBC 853-1050 (ISSR marker, green oval). Chromosome spreads (right) show hybridization signals of M1C and M6B (red) and UBC 853-1050 (green) on the same medium length chromosome pair
7. Genomic in situ hybridization (GISH) technique to distinguish between E. oleifera and E. guineensis chromosomes in interspecific hybrids

This technique assists breeders involved in interspecific breeding programmes where parental genome compositions can be determined and selection of interspecific hybrid palms of interest be done.

Figure 17. (A) The interphase nuclei of the OxG hybrids had groups of chromosomes from both parental genomes in discrete, non-intermixed domains indicating non-random organization of the nucleus. (B) F1 hybrids showed clear differentiation between the 16 E. oleifera (yellow) and 16 E. guineensis (red) chromosomes

CONCLUSION

As illustrated in the limited examples above, it is proven that cytogenetical analysis is a powerful classical tool that can be used by itself or in combination with molecular cytogenetics to enable efficient research to be done at the chromosomal level. Molecular cytogenetics, which allows the linking of molecular biology with cytogenetics, has revolutionized the investigation of structure, function, organization and evolution of genes and genomes by fluorescent or genomic in situ hybridization (FISH or GISH) techniques. These tools in turn provide platforms for wholistic basic research on genome studies, cytogenetic mapping for crop improvement, and quality control on the ortets used and ramets produced.

REFERENCES