FLOW CYTOMETRY APPLICATIONS

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MPOB has purchased a FACS Calibur Flow Cytometer (Becton Dickinson, USA) in 2003 (*Figure 1*). It is equipped with an argon ion laser for analysis of the fluorescent intensity of nuclei using the CellQuest software. Flow cytometry (FCM) is a rapid and convenient technique that allows accurate determination of (1) nuclear DNA content or genome size in plants, and (2) the ploidy level.

DNA CONTENT ESTIMATION

Knowledge of the DNA content (genome size) is important in many areas of research ranging from evolutionary studies to genome mapping. To determine the nuclear DNA content in absolute units, the fluorescence intensity of the sample nuclei is compared with the fluorescence intensity of nuclei isolated from a species with known nuclear genome size, for example, *Glycine max* cv. Polanka (2C=2.5 pg). The genomic DNA content reported by Rival *et al.* (1997) using flow cytometry for *E. guineensis* (*tenera*) was 2C = 3.786 ± 0.125 pg. MPOB has found that the 2C DNA content for average *E. guineensis* to be 3.86 ± 0.26 pg. For fruit type *dura* (D), it is 4.10 ± 0.20, for *pisifera* (P) 3.64 ± 0.28, and for *tenera* (DxP) 3.83 ± 0.31 pg. *E. oleifera* (Suriname) has 2C = 2.08 ± 0.04 pg and OG hybrids 2C = 4.16 ± 0.32 pg.

PLOIDY ANALYSIS

Ploidy manipulation is a valuable tool in plant improvement, as has already been demonstrated in *Solanum*, citrus and azaleas. Ploidy is induced for several reasons in, for example, citrus. Tetraploid parents are produced to create seedless triploids by crossing 4n with 2n parents. In herbal plants, such as *Scutellaria* and *Artemisia*, tetraploidy increases their contents of the secondary metabolites, baicalin and artemisinin, respectively. In azalea and pomegranate, chromosome doubling is used to obtain new ornamental characteristics. Besides, polyploidy often generates variants that may contain useful characteristics, and by doubling the gene products, polyploids also provide a wider germplasm base for breeding.

Colchicine and the herbicide, oryzalin, have been successfully applied to induce polyploids. These chemicals act by binding to the tubulin dimers so

Figure 1. FACS Calibur (Becton Dickinson, USA) Flow Cytometer (yellow arrow). The monitor on the left is displaying fluorescent intensity histogram peaks of nuclei stained with propidium iodide captured by the CellQuest software.

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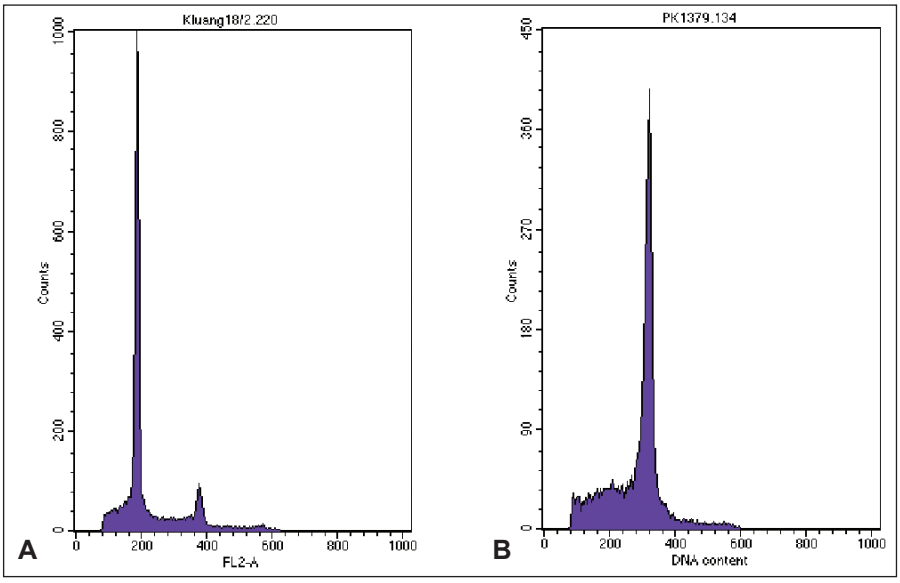

Figure 2. Examples of fluorescent intensity peaks for (A) standard Glycine max *cv. Polanka (2n), and (B)* E. guineensis *(2n).*

that microtubules fail to form, and, consequently too no spindle fibres during meiosis or mitosis. The need to increase variability in the narrow genetic base of Malaysian oil palm has driven MPOB researchers to employ polyploidy, and flow cytometry to efficiently determine the ploidy levels of seedlings exposed to colchicine and oryzalin. In MPOB's study, colchicine was more efficient than oryzalin in inducing chromosome doubling from 2n to 4n.

BENEFITS

Flow cytometry is an efficient and fast method to estimate the DNA content and analyse the ploidy level of oil palm or any organism. It is a useful tool for research ranging from evolutionary studies to genome mapping and ploidy manipulation.

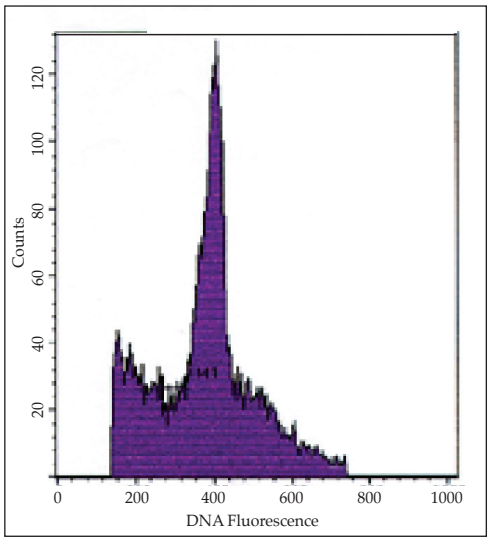

Figure 3a. Example fluorescent intensity histogram peak for E. guineensis *(tetraploid, 4n).*

Figure 3b. Colchicine-treated tetraploid seedling (left) and controls.

COST

Consultation fee per analysis (DNA content estimation or ploidy analysis) is RM 5000. This includes training to use the FACS Calibur Flow Cytometer, method to prepare leaf samples for Flow Cytometry, use of the CellQuest software and ANOVA for the data, interpretation of the results and 20 man-hours assistance per engagement of service. The bench and chemical fees are RM 1000 per the 25-flow cytometry analysis.

For more information kindly contact:

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