IMAGE CYTOMETRY (ICM): GENETIC ANALYSIS OF OIL PALM CALLI AND SUSPENSION CULTURES

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For oil palm calli and suspension cultures, genetic variation analysis, estimation of genome size and observation of nuclei activity is not possible via flow cytometry due to the low population of nuclei present. Hence, image cytometry or ICM method was developed to address this issue. The approach is to quantitate the chromogenic areas of the black and white nuclei images followed by calculation of integrative optical density (IOD) via image analysis software (Oberholzer *et al.*, 1996). It is important that all sample preparations and image processing are carried out in a consistent manner at all times.

MPOB has developed an ICM technique using PAX-it image database and analysis software. The reference standard used in this study is a *tenera* seedling with nuclear DNA content of 2C=3.53 pg measured by flow cytometry, while the root tips meristematic nuclei were used as standard for image cytometry analysis. Two reference standards are required to estimate the genome size of calli samples analysed. Reference standard nuclear DNA content must be known before the genome estimation of sample can be done (Vilhar and Dermastia, 2002).

MATERIALS AND METHODS

Root tips of *tenera* seedling (standard) and the suspension calli were fixed in 4% formaldehyde and stored at 4°C until needed. The image cytometry procedure was modified from the method of Greilhuber and Temsch (2001). Firstly, the set of calli sample and root tips of the standard were rinsed several times in distilled water. Then the materials were acid hydrolysed in 5N HCl for 90 min at 20°C. The materials were then rinsed in ice-cold distilled water for approximately 10 min (5 x 2 min) and immersed in Schiff's reagent at room temperature (1.5 hr) or 4°C (12-15 hr or overnight). Next, they were rinsed in sulphide water for 3x5 min, followed by 3x10 min each. After a short rinse in distilled water, the materials were squashed separately with 45% acetic acid within 3 to 5 min. Slides were frozen to remove coverslips followed by dehydration in ethanol series 50%, 70%, 90% and 96% for 10 min each. The slides were then mounted with antifade media (Citifluor) and observed using epifluorescent microscopy. The images of the cells observed under Feulgen fluorescence (red) were captured using the PAXCam camera and the black and white images were saved in bitmap format. Images were captured under constant illumination, exposure and magnification conditions. IOD values of the nuclei were measured using the PAX-it software for each nucleus. Histogram charts of IOD associated with the number of nuclei were plotted using Microsoft Excel to observe the pattern of nuclei activity.

RESULTS AND DISCUSSION

Standard: Estimation of Genome Size Using *Tenera* Leaves via Flow Cytometry (FCM) and Nuclei of Root Tip Meristems via Image Cytometry (ICM)

Tenera seedling genome size estimation via FCM is 2C = 3.53 pg while via ICM the 2C IOD peak is at 0.09 arbitrary units (au).

Sample: Suspension Calli PL 168

The 2C IOD peak of *tenera* seedling standard is 0.09 au (2C=3.53 pg) while the 2C IOD peak of suspension calli PL 168 is 0.12 au. Using simple calculation the estimated genome size of PL 168 is 2C=4.71 pg.

Image Cytometry Application

ICM has been mostly applied in medical research and only recently being utilised in plants (Vilhar and Dermastia, 2002). Vilhar *et al.* (2001) has used *Pisum sativum* as a calibration standard (2C=8.84 pg) and applied the ICM technique to estimate nuclear DNA content of eight plant species such

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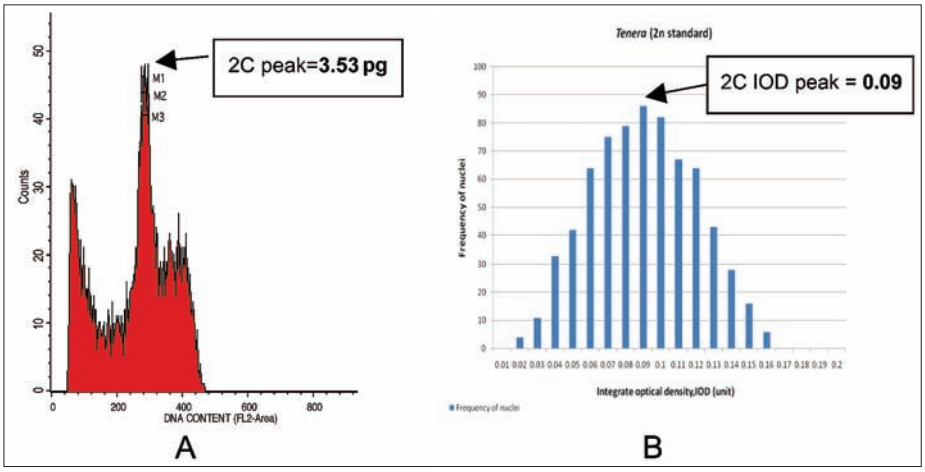

Figure 1. Shows (A) standard tenera *FCM 2C peak = 3.53 pg (arrow) and (B)* tenera *root tips ICM 2C IOD peak as 0.09 au (arrow).*

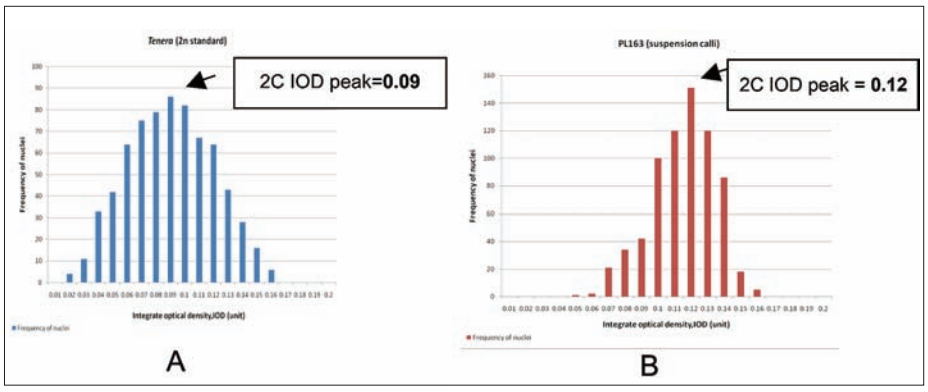

Figure 2. Shows (A) standard tenera *ICM 2C peak = 0.09 au (arrow) and (B) suspension calli PL168 ICM 2C peak as 0.12 au (arrow).*

as *Allium cepa*, *Secale cereal*, *Vicia faba*, *Hordeum vulgare*, *Zea mays*, *Glycine max*, *Raphanus sativus* and *Arabidopsis thaliana*. ICM produced accurate and reproducible results.

The ICM method developed using PAX-it software has made it possible for DNA content of materials such as calli to be estimated that otherwise could not be determined by flow cytometry. As such, this benefits the researchers and oil palm industry members that are interested in estimating the genome size of calli and studying the genetic variation during oil palm micropropagation.

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