

OIL PALM HAPLOID TECHNOLOGY: SCREENING FOR NATURALLY OCCURRING HAPLOIDS

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Haploids are individuals with gametic chromosome number (n) or having a single set of chromosomes. Knowledge of the haploid number is useful in breeding and genetic studies. Haploids are used to produce double haploids ($2n$) where the chromosome number is doubled using chemicals. Chemicals such as colchicine or oryzalin interfere with tubulin formation during mitosis. Double haploid (DH) facilitate the following:

- production of completely homozygous plants in a single generation;
- disclosure, segregation and fixation of the genetic variation of an F1 generation;
- genetic analysis where efficient analysis and combination of multiple (recessive) genetic traits can be done;
- direct fixation of new target genes in a pure line for release as new variety, especially for self-pollinated crops;
- rapid production of F1 hybrid parent lines;
- genetic manipulation; and
- studies in genetic mapping and genomics, such as:
 - a. molecular marker maps or marker assisted breeding;
 - b. quantitative trait loci (QTL) mapping;
 - c. bulked segregation analysis; and
 - d. improved backcrosses.

OBJECTIVE

The objective is to screen the culled or abnormal oil palm germinated seeds for naturally occurring haploids via flow cytometry (FCM).

METHODOLOGY

Sorting of Abnormal Germinated Seeds

In seed production, there are at least 13 criteria of abnormal germinated seeds. Most of these are based on abnormalities of the plumule and radicle

(Figure 1), such as stunted plumule, radicle or both.

Planting of Abnormal Germinated Seeds

The abnormal germinated seeds were planted in jiffy pots according to their abnormality category and arranged in plastic baskets on racks in the nursery (Figure 2a). After three months, the seedlings were analysed by FCM to determine their ploidy level (Figure 2b).

Flow Cytometry Analysis

About 1 cm x 1 cm leaf samples of abnormal seedlings were cut into small pieces and placed into a 50 μ m Medicon tissue homogeniser containing LBO1 buffer supplemented with 50 μ g PI (propidium iodide) and 50 μ g RNaseA. The samples were blended using Medimachine for 3 to 5 min, filtered into a 10 ml falcon tube and incubated at 4°C for 24 hr followed by FCM analysis. The fluorescence intensity of each sample was measured by FACSCalibur flow cytometer equipped with 15 mW argon ion laser at 488 nm. Histograms were recorded over 1024 channels and for leaf samples 15 000 events were captured. The ploidy level was determined when the haploids exhibited fluorescent histogram peak at channel 150 -160. Finally, diploids were discarded and haploids maintained for further confirmation via 18S-25S ribosomal DNA fluorescent *in situ* hybridisation (rDNA-FISH) or chromosome counting.

RESEARCH FINDINGS

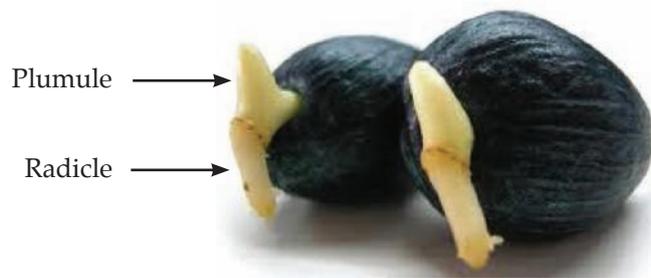
Screening abnormal seedlings via FCM proved to be a useful method even though the frequency of naturally occurring haploids was less than 0.5%. Figure 3a shows haploid plants. Figure 3b shows fluorescent intensity histogram peak occurring at channel ~160 of a sample derived from a germinated seed with stunted plumule.

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(a) Normal germinated seeds



Stunted plumule



Colour of plumule changed



Growth of twin seedlings (V shape)



Stunted radicle



Ratio of plumule and radicle exceed 1:1



Doubleton with stunted plumule/radicle/both



Radicle is too thin



Plumule and radicle in 90° position



Branched plumule



Branched radicle



Stunted plumule and radicle



Colour of radicle or radicle and plumule changed

(b) Abnormal germinated seeds

Figure 1. Morphology of oil palm germinated seeds: (a) normal and (b) abnormal.



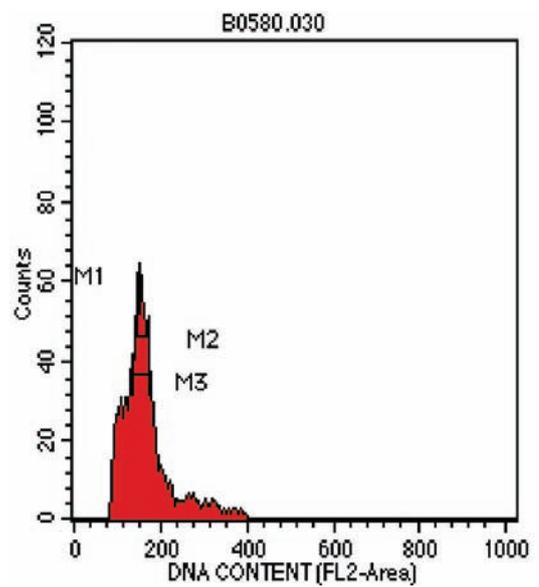
Figure 2a. Abnormal germinated seeds planted in jiffy pots arranged in plastic baskets.



Figure 2b. Three-month-old abnormal seedlings (left baskets).



a



b

Figure 3. (a) Naturally occurring haploid plants obtained from flow cytometry screening of abnormal seedlings and (b) fluorescent intensity histogram peak of a haploid at channel ~160.

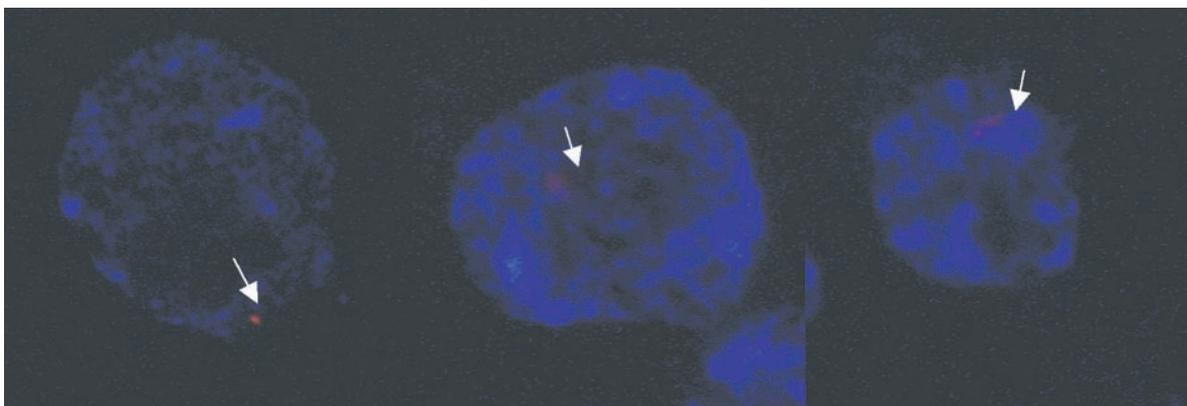


Figure 4. Interphase nuclei exhibiting one rDNA signal (arrow) indicating the sample is a haploid.

Ribosomal DNA (18S-25S) Fluorescent *in situ* Hybridisation (rDNA-FISH)

FISH of rDNA was performed on the nuclei of putative haploids to confirm the ploidy level. An interphase haploid nucleus will show one major signal, diploid will exhibit two; triploid will exhibit three major signals. *Figure 4* shows the interphase nuclei exhibiting one rDNA signal hence indicating the sample is a haploid. The first nucleus showed one intense signal, while the second and third nuclei showed a diffused signal indicating that the respective nucleolar organiser region (NOR) was actively transcribing.

CONCLUSION

High throughput FCM is a practical method to screen naturally occurring haploids in abnormal oil palm germinated seeds.

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