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# Suitability of RAPD analysis for the detection of somaclonal variants in oil palm (*Elaeis guineensis* Jacq)

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## Abstract

Random amplified polymorphic DNA (RAPD) analysis using arbitrary 10-mer oligonucleotide primers was employed in order to investigate the genetic fidelity of somatic embryogenesis-derived regenerants of oil palm (*Elaeis guineensis* Jacq). Clonal palms bearing the 'mantled' phenotype were identified in the field and the ability of RAPD markers to distinguish these variants from palms of the normal type was assessed. Of the 387 arbitrary primers used, 259 (67%) were successfully used to amplify oil palm DNA genomic fragments with consistently reproducible banding. Of these 387 primers, 73 (19%) primers enabled the identification of polymorphism between clones. No intraclonal variability and no differences between mother palms and regenerants could be identified using the total number of markers scored (8900). Twenty-four of these 73 primers were chosen for use in a larger experiment aimed at comparing, first, the mother palm genome with that of its clonal offspring and, second, true-to-type and variant regenerants. Thus, the regeneration protocol based on somatic embryogenesis set up for oil palm clonal propagation does not induce any gross genetic changes. The results obtained revealed however, that the RAPD approach is not suitable for the detection of the mantled variant phenotype. The use of RAPD markers for the detection of somaclonal variation in oil palm is discussed.

**Key words:** *Elaeis guineensis* — mantled fruit — RAPD marker — somaclonal variation — somatic embryogenesis

Clonal propagation of oil palm (*Elaeis guineensis* Jacq) through somatic embryogenesis has been practised for more than 10 years by several research groups, and has led to the production of several millions of clonal plantlets. The technique has been proven by field performances on more than 2500 ha of clonal plantations (see Duval et al. 1995 for a review).

Evaluation in the field has, however, revealed the occurrence of a small percentage (*c.* 5%) of variant palms which show an abnormal flower development. This character, originally referred to as 'mantled' by Corley et al. (1986) has been characterized as a feminization of the male parts of flowers from both sexes. The mantled abnormality can result in complete sterility of the variant palm in the most severe cases. The early detection of mantled-type somaclonal variants is thus critical in oil palm clonal micropropagation.

The origins, causes and breeding applications of somaclonal variation have been studied intensively (Larkin and Scowcroft 1981, Karp 1991, Kaeppler and Phillips 1994). The use of molecular markers is becoming widespread for the identification of somaclonal variants and the assessment of micropropagation or *in vitro* regeneration protocols (Brown et al. 1991, Vallés et al. 1993, Heinze and Schmidt 1995, Wallner et al. 1996). For this purpose, randomly amplified polymorphic DNA (RAPD) appears particularly suitable on account of its ability to analyse DNA variation at many loci using small amounts of tissue (Williams et al. 1990). RAPD markers have proved useful for a number of purposes, such as the evaluation of genetic integrity during tissue culture (Isabel et al. 1993, Taylor et al. 1995), the identification of clonal plant material (Castiglione et al. 1993, Rani et al. 1995, Villordon and LaBonte 1995) and the detection of somaclonal variants (Munthali et al. 1996).

In oil palm, RAPD markers have been successfully employed for the determination of genetic variation among different accessions of germplasm (Shah et al. 1994). The aims of the work described here were: (1) to detect the occurrence of gross genetic variation during oil palm micropropagation through somatic embryogenesis; and (2) to assess the suitability of RAPD for the detection of mantled variants among regenerant population. For these purposes, a large number of 10-mer primers (over 350) have been screened for their utility in the detection of polymorphism. The further characterization of regenerant palms was performed using 24 selected primers. DNA polymorphism was investigated on 68 different palms through the analysis of mother palm/regenerants and true-to-type/variant regenerants from several clones.

## Materials and Methods

**Plant material:** Elite mother palms and regenerants of *E. guineensis* Jacq obtained through *in vitro* somatic embryogenesis were sampled at the IDEFOR-DPO LaMé Research Station in Côte d'Ivoire (West Africa). Plant material consisted of 3–7-year-old palms of *dura* × *pisifera* hybrid origin. Leaf samples consisted of *c.* 20 leaflets taken from the basal part of the (F + 1) frond. Because of estate management contingencies it was unfortunately not possible to sample the mother palm and its true-to-type/variant regenerants from the same clone. The screening of each PCR primer was performed using four DNA samples, originating from a variant palm and a true-to-type palm of two different clonal lines, namely LMC51 (pedigree: L2T × D8D) and LMC63 (L2T × L269D). Comparison between mother palms and regenerants was carried out on four different true-to-type clonal palms and their corresponding mother palm in four different clonal lines:

- LMC174: (D3D × D10D) × (L2T × L2T);
- LMC238: (D5D × D5D) × (L2T × (L2T × Si10T));
- LMC246: (D115D × D115D) × (L2T × L2T);
- LMC343: (D115D × D115D × (L2T × L2T).

In order to compare true-to-type and variant regenerants originating



from the same clonal line, eight palms (four bearing the normal phenotype and four variants) were sampled from four different clonal lines: LMC03 (L16T × D3D), LMC51, LMC52 (L3D × L2T) and LMC63. Normal and variant clonal palms derived from the same culture batches differed only in their phenotypes; the *in vitro* treatments that they had previously experienced were identical.

**Tissue culture:** Clonal oil palm plantlets were obtained from immature leaves through somatic embryogenesis (Pannetier et al. 1981, Duval et al. 1995). This micropropagation procedure allows the survival of the mother palm after sampling. It was thus possible to take leaf samples of the latter following the field monitoring of its clonal offspring (at least at 4 years after the initiation of tissue culture).

**DNA isolation:** DNA was isolated following a two-step method involving the extraction of genomic DNA from nuclei. Freeze-dried leaves (*c.* 2g dry weight) were ground to fine powder using a domestic coffee grinder. The powder was resuspended in 200 ml (1 ml/10 mg dry weight) of extraction buffer (0.35 M sorbitol, 0.1 M Tris, 5 mM EDTA, 0.5% (w/v) $Na(SO_2)_2$) and filtered through a nylon cloth. Following a centrifugation at 3000 *g* for 20 min, the pellet containing the nuclei was resuspended in 120 ml of lysis buffer (0.1 M Tris, 1.25 M NaCl, 0.02 M EDTA, 4% (w/v) MATAB, pH 8.0). After incubation for 4 h at 65°C in a water bath, with occasional inversion, the total volume of lysate was adjusted to 200 ml with a solution of 24:1 (v/v) chloroform–isoamyl alcohol. After agitation, the homogenate was centrifuged at 3000 *g* for 10 min. The extraction with chloroform–isoamyl alcohol was repeated and the aqueous fraction (*c.* 25 ml) then incubated with 200 µl of a 10 mg/ml RNAse A solution (from bovine pancreas, Boehringer Mannheim, Germany) for 1 h at 37°C. Total DNA was precipitated with 25 ml isopropanol. The DNA precipitate was rinsed in 70% (v/v) ethanol, then vacuum-dried. The dried DNA pellet was resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). DNA was quantified by gel electrophoresis along with known amounts of standard size markers (phage DNA double digested with *Hin*dIII and *Eco*RI). Extraction of genomic DNA from freeze-dried oil palm leaves yielded 250–500 µg/g dry wt. This result shows a significant improvement in the technique if compared with the yields previously reported for oil palm leaves (20 µg DNA/g dry wt, as reported by Shah and Parveez 1992).

**DNA amplification:** A series of 387 arbitrary decamer oligonucleotides purchased from Operon Technologies (CA, USA) were used for the amplification of random DNA sequences. Conditions for DNA amplification and electrophoretic resolution of PCR products have been described previously (Lashermes et al. 1996).

## Results

### Comparative RAPD analysis of normal and variant regenerants

A total of 387 arbitrary 10-mer oligonucleotide sequences were first screened for their amplifying ability using four oil palm DNA samples (one normal-type and one variant palm from two different clonal lines). Of the 387 primers used, 259 (67%) successfully amplified oil palm DNA with consistently reproducible banding. Of these, 73 primers (19%) were able to distinguish polymorphism between clonal lines. No polymorphism related to the mantled variation could be detected (Table 1).

Table 1: Screening of RAPD primers using nuclear DNA samples from four different oil palms: one true-to-type and one variant palm from each of two different clonal lines: LMC51 and LMC63

| Series | No. of primers | Primers giving exploitable amplification products | Primers able to reveal interclonal polymorphism | Polymorphism related to the mantled variation |
|---|---|---|---|---|
| OPA | 19 | A1, A2, A4, A6, A7, A8, A9, A10, A11, A12, A13, A14, A15, A16, A17 A18, A19, A20 | A8, A9, A10, A17, A19, A20 | None |
| OPB | 20 | B1, B2, B3, B5, B6, B7, B8, B9, B10, B11, B12, B13, B14, B15, B16, B17, B18, B19, B20 | B3, B7, B9, B14, B20 | None |
| OPC | 20 | C1, C3, C4, C6, C7, C8, C9, C10, C11, C14, C16, C17 | C8, C16 | None |
| OPD | 20 | D1, D3, D4, D5, D6, D7, D9, D10, D11, D12, D15, D16, D20 | D1, D3, D13, D15 | None |
| OPE | 20 | E1, E2, E3, E4, E5, E6, E8, E9, E10, E11, E12, E13, E14, E15, E16, E17, E18, E19, E20 | E11, E13, E16, E19 | None |
| OPF | 20 | F4, F5, F6, F7, F8, F9, F10, F11, F12, F13, F14, F15, F16, F17, F18, F19, F20 | F4, F6, F9, F11 | None |
| OPG | 20 | G4, G5, G6, G8, G9, G10, G11, G12, G13, G14, G15, G16, G18, G19, G20 | G11, G15, G19, G20 | None |
| OPI | 19 | I5, I8, I10, I15, I19 | None | None |
| OPJ | 20 | J3, J4, J6, J8, J12, J15 | J1, J4, J12 | None |
| OPK | 20 | K1, K3, K5, K6, K8, K9, K10, K13, K14, K16, K17, K18, L20 | K5, K8, K13, K18 | None |
| OPL | 20 | L6, L4, L9, L12, L13, L16, L17, L18, L20 | L4, L9, L16, L17, L18, L20 | None |
| OPM | 20 | M2, M3, M6, M7, M9, M10, M11, M13, M14, M15, M16, M19 | M3, M7, M9, M14, M19 | None |
| OPN | 20 | N1, N2, N3, N7, N10, N11, N12, N13, N14, N15, N19, N20 | N7, N10, N11, N19 | None |
| OPP | 18 | P2, P5, P6, P8, P9, P10, P11, P12, P17, P18, P20 | P5, P17, P20 | None |
| OPQ | 17 | Q4, Q5, Q6, Q8, Q9, Q10, Q11, Q12, Q13, Q14, Q15, Q17, Q19, Q20 | Q8, Q11, Q19 | None |
| OPR | 18 | R1, R3, R4, R5, R6, R7, R9, R10, R11, R12, R13, R14, R15, R16, R17, R19 | R17 | None |
| OPS | 18 | S1, S2, S4, S5, S6, S10, S13, S14, S15 | S1, S4 | None |
| OPX | 20 | X3, X4, X5, X6, X10, X16, X18, X20 | X3, X4, X20 | None |
| OPY | 18 | Y2, Y3, Y4, Y5, Y6, Y7, Y8, Y9, Y10, Y15, Y16, Y17 | Y2, Y3, Y10, Y15 | None |
| OPZ | 20 | Z1, Z3, Z4, Z5, Z6, Z7, Z8, Z10, Z11, Z12, Z13, Z14, Z15, Z16, Z17, Z18, Z19, Z20 | Z1, Z3, Z8, Z11, Z18. Z19, Z20 | None |
| Total | 387 | 259 | 73 | None |

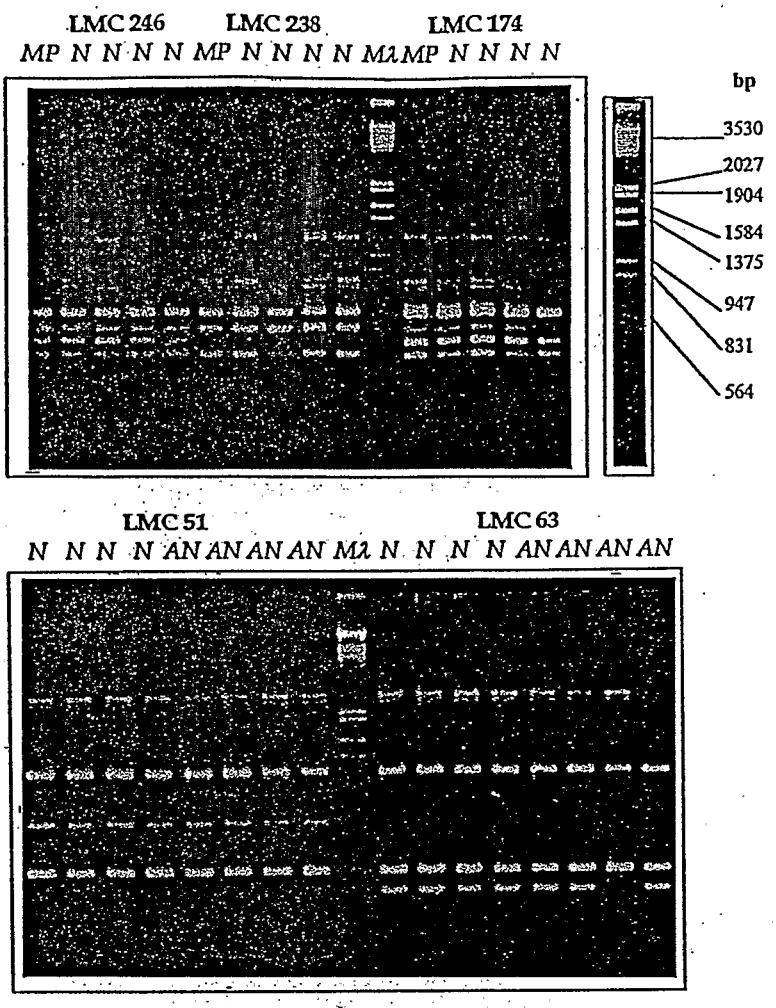


Fig. 1: Gel electrophoresis of RAPD fragments obtained in *Elaeis guineensis*. a. Comparison between mother palm (MP) and normal regenerants (*N*), originating from clones. b. Comparison between normal (N) and abnormal regenerants (*AN*), originating from clones LMC51 and LMC63. Primer was OPJ04

Primers produced an average of 5.4 amplification products, ranging from 500 to 2530 bp, with an average size of 1020 bp. For a larger study, 24 primers were selected for their reproducibility and clarity of results and performed RAPD on a total of 32 palms (four normal and four mantled regenerants from four clones). For all of the 24 primers tested, amplification products were monomorphic throughout all the somatic embryo-derived palms of the same clonal line regardless of whether their phenotype was variant or true-to-type (Fig. 1a).

**Comparative RAPD analysis of mother palms and regenerants**

For the four clonal lines examined, PCR amplification products obtained with the four somatic embryo-derived palms were compared with those of their original mother palm. With all primers used, a complete coincidence of RAPD profiles was found between the original palm and its four regenerants, for the four clones considered. RAPD profiles were polymorphic from one clonal line to another, thus confirming the results previously obtained on four other different clones (Fig. 1b).

## Discussion

RAPD analysis efficiently differentiated oil palm clones. These results will be useful for further studies such as clonal identification, the monitoring of progenies in seed-production programmes and in genetic mapping, thus complementing those previously obtained by Shah et al. (1994). RAPD analysis has failed to reveal any polymorphism associated with somaclonal variation in regenerated oil palm clonal plantlets. Nevertheless, somaclonal variants were clearly identified in the field through their phenotypic characteristics (Corley et al. 1986), but were not detected by RAPD analysis. Our results indicate that gross genetic change is rare or absent in the tissue culture protocol under investigation. RAPD analysis proved suitable for detecting gross genetic changes occurring in sugarcane tissues subjected to prolonged *in vitro* culture, but was not sufficiently sensitive to detect smaller changes during genetic transformation (Taylor et al. 1995). Similarly, Vallès et al. (1993) did not detect any variants in *Lolium* and *Festuca* regenerants by RAPD analysis. In a study on clonal fidelity using RAPD in spruce, Heinze and Schmidt (1995) concluded that a low frequency of genetic instability was present in the population of somatic embryo-derived plantlets. Conversely, Brown et al. (1991) assessed the RAPD approach as being very efficient for the study of DNA polymorphism in *Triticum tauschii*, avoiding the need for complex and expensive methods for the analysis of somaclonal variation. Assessing RAPD for the detection of somaclonal variants of beet, Munthali et al. (1996) found the overall frequency of somaclonal polymorphism (0.05%) to be similar to the frequencies reported using other techniques, such as isozymes or RFLP.

In our experimental conditions, involving 259 primers, the total size of amplification products scored for polymorphism averaged roughly 0.04% of the oil palm genome size of $3.6 \times 10^9$ bp (Rival et al. 1997). The probability of detecting

polymorphisms resulting from macroevents (deletions, insertions, substitutions) at the DNA level within an amplified region is thus rather low.

Nevertheless, the total number of bands scored in this study as a whole (including both studies of mother palm/regenerants and those of normal/abnormal regenerants) reached 8813 (68 palms × 5.4 bands × 24 primers). This number was much higher than the those published in other studies, indicating that the frequency of polymorphism in the somatic embryo-derived material is very low. It should be much lower than 0.05%, if compared with the results presented by Munthali et al. (1996) who found three somaclonal polymorphisms in 5607 scored RAPD bands obtained from 120 regenerants of sugar beet. Similarly, Devaux et al. (1993) examined 5340 RAPD bands in *Hordeum* without finding any polymorphism in embryogenically derived material. Isabel et al. (1993) detected no polymorphism in *Picea* regenerants after scoring 900 RAPD markers.

Future work will focus on the study of mechanisms involved in differential gene expression in true-to-type and variant regenerants, in relation to genomic DNA methylation.

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**Titre Original :** Suitability of RAPD analysis for the detection of somaclonal variants in oil palm *(Elaeis guineensis* Jacq)

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**Titre en Français :** Pertinence de l'analyse RAPD pour la détection de variants somaclonaux chez le palmier à huile (*Elaeis guineensis* Jacq.)

**Résumé en français :** Une analyse RAPD a été réalisée à l'aide d 'amorces arbitraires de 10 nucléotides, dans le but d'étudier la conformité génétique de régénérants de palmier à huile (*Elaeis guineensis* Jacq) issus d'embryogenèse somatique. Des palmiers régénérants présentant le phénotype « mantled » ont été identifiés au champ, et la capacité des marqueurs RAPD à mettre en évidence ces variants a été vérifiée. Sur les 387 amorces testées, 259 (67%) ont permis d'amplifier avec succès les fragments d'ADN génomique de palmier à huile avec des profils électrophorétiques répétables. Sur ces 387 amorces, 73 (19%) ont permis d'identifier un polymorphisme entre clones. A partir de ces 73 amorces, 24 ont été sélectionnées pour une expérimentation à plus grande échelle, destinée à comparer, dans un premier temps le génome des têtes de clone avec celui des régénérants clonaux qui en étaient issus, puis, dans u deuxième temps les régénérants normaux et variants. En criblant les 8900 marqueurs générés, nous n'avons pu identifier variabilité intraclonale ni de différences entre tête de clone et régénérants. Il apparaît donc que le protocole de micropropagation clonale employé pour le palmier à huile, basé sur l'embryogenèse somatique, n'induit pas d'importants bouleversements génomiques. Nos résultats ont permis de montrer que l'approche RAPD n'est pas appropriée à la détection du phénotype variant « mantled ». Dans cet article, l'emploi des marqueurs RAPD pour la mise en évidence de variations somaclonales chez le palmier à huile est discutée, et des approches moléculaires alternatives sont proposées.

**Mots-clés:** *Elaeis guineensis* - fruit « mantled » - marqueur RAPD - embryogenèse somatique - somaclonal variation