

Study of endogenous cytokinin levels associated with an anomaly in floral morphogenesis of *Elaeis*

Isabelle Besse, Thierry Beulé, Jean-Luc Verdeil,
Yves Duval
In collaboration with Régis Maldiney, Bruno Sotta,
Emile Miginiac

In oil palm (*Elaeis guineensis* Jacq.), vegetative multiplication by somatic embryogenesis from callus has been carried out on a large scale in laboratories in Africa and East Asia since the beginning of the eighties. Two different types of calli can be used to obtain somatic embryos: compact nodular callus (CNC), which are yellow in color and made up of meristemic tissue organized in pseudocambium (SCHWENDIMAN *et al.*, 1988); and rapid-growth calli (RGC), which are friable, white in color and made up of a meristemic clump and tissue in the course of degenerescence; RGC occur spontaneously on CNC (HANOWER and PANNETIER, 1982).

Experimental planting has shown that trees regenerated from somatic embryos may present an anomaly in floral morphogenesis which is specifically induced by *in vitro* culture. The anomaly, which is commonly referred to as "mantled" anomaly, is only observed in trees produced by cloning. It corresponds to a disturbance in sexualization and is characterized by feminization of the male organs in flowers of both sexes. Expression of the anomaly in the field varies in intensity. Observations of more than 20 000 individuals produced from 92 clones showed that only 5% of the trees regenerated from CNC (the percentage varied with the clones) carried the anomaly, whereas all palm trees regenerated from RGC were abnormal (DUVAL *et al.*, 1988).

These observations suggest growth regulators are involved in the phenomenon. An original approach was used to determine the mode of expression of the anomaly by measuring phytohormones in the different tissues. In a preliminary step, hormone characterization of the inflorescences of two clones, C5 and C6, was carried out in order to establish correlations between the disturbance in sexual differentiation and possible changes in hormone metabolism.

Endogenous phytohormone levels were measured using an ELISA-type immunoenzymatic method (MALDINEY *et al.*, 1986). Abnormal inflorescences were characterized by lower levels of cytokinins than normal inflorescences and the greater the degree of the anomaly, the lower the level of cytokinins within the inflorescence (Figure 1).

Analysis of four different clones (C1, C2, C3, C4) showed that endogenous cytokinin levels depend on the type of callus. In CNC only two cytokinins were detected: 9-glucoside zeatin ([9G]Z) and 9-riboside zeatin ([9R]Z). Irrespective of the clone, levels of [9G]Z were always higher than those of [9R]Z.

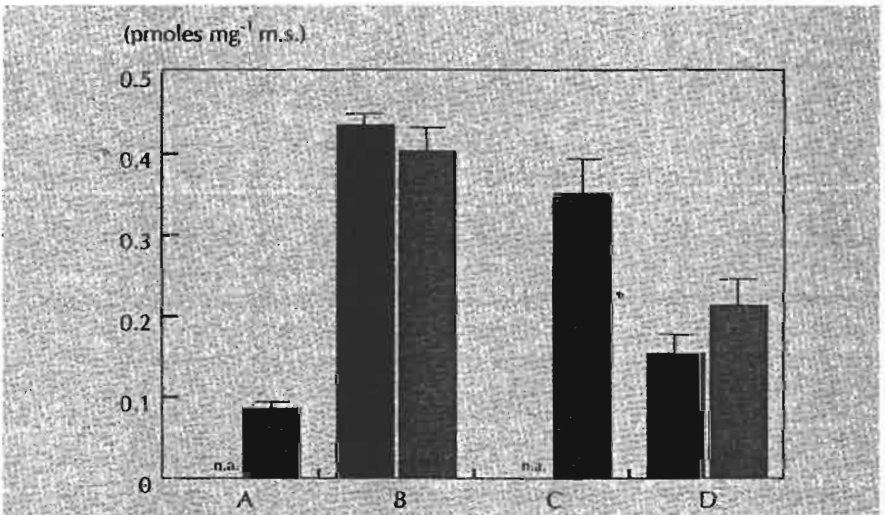


Figure 1. Comparison of levels of [9R]Z in different inflorescences of the clones C5 (■) and C6 (▒). These results are the means of five repetitions (\pm standard deviation).

A: normal male inflorescences of a palm tree from CNC;

B: normal female inflorescences of a palm tree from CNC;

C: partly abnormal female inflorescences of a palm tree from CNC;

D: totally abnormal female inflorescences of a palm tree from RGC;

n.a. Not assessed.

However, cytokinin levels were much lower in RGC than in CNC on standard medium (Figures 2 and 3). Moreover in RGC, during the course of a multiplication cycle, cytokinin levels were always below the threshold of detection of this method, while in CNC cytokinins could always be detected (Figure 4).

Prospects

The disturbance in zeatin cytokinin metabolism in RGC was also observed in flowers of plants regenerated from this type of callus. These results enable us to define the determinism of this anomaly with more precision and this study, which opens the way for further studies of somaclonal variations, should finally lead to the control of trueness-to-type in oil palms regenerated by somatic embryogenesis.

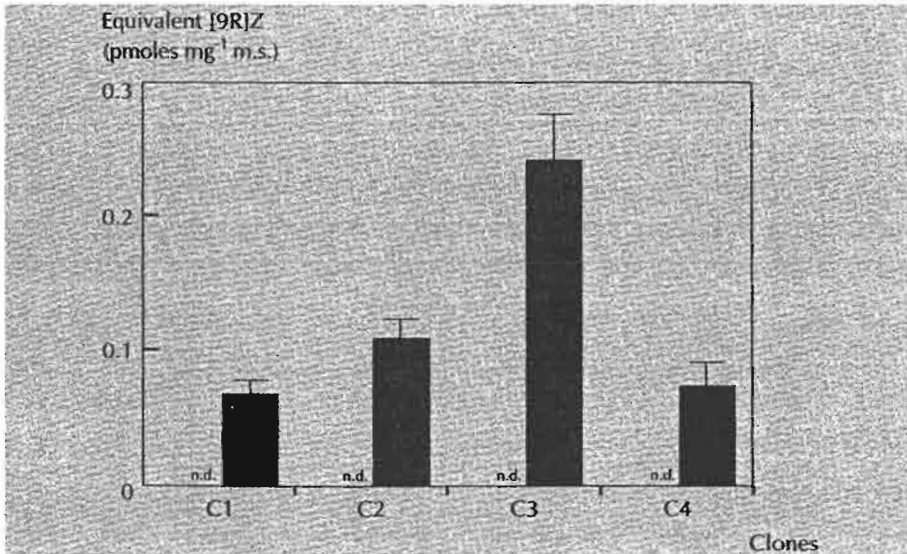


Figure 2. Comparison of levels of [9G]Z in CNC (□) and RGC (■) of four clones cultured on standard medium (MI 58).

Calli are harvested 10 days after transfer on a new medium.

These values are distribution means (\pm standard deviation).

n.d. Not detected.

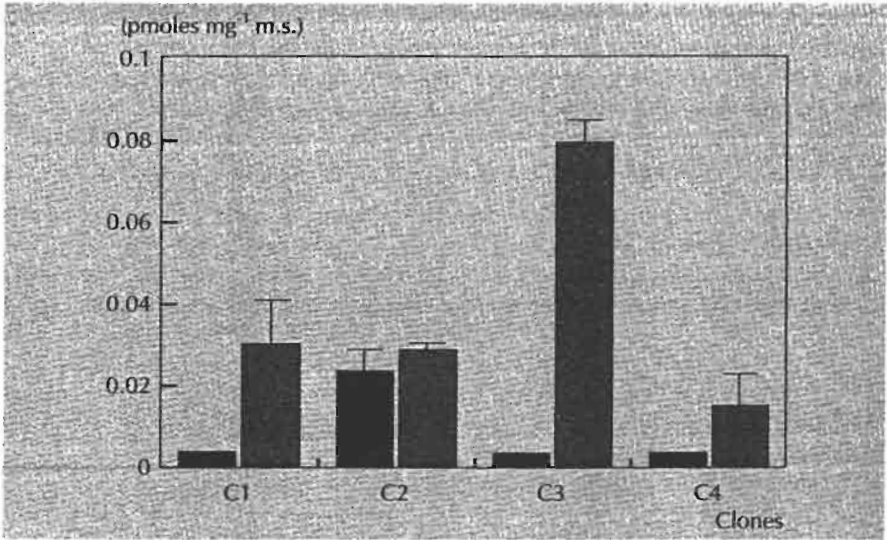


Figure 3. Comparison of levels of [9R]Z in CNC (▨) and RGC (■) of four clones cultured on standard medium (MI 58).

Calli are harvested 10 days after transfer on a new medium. These values are distribution means (\pm standard deviation).

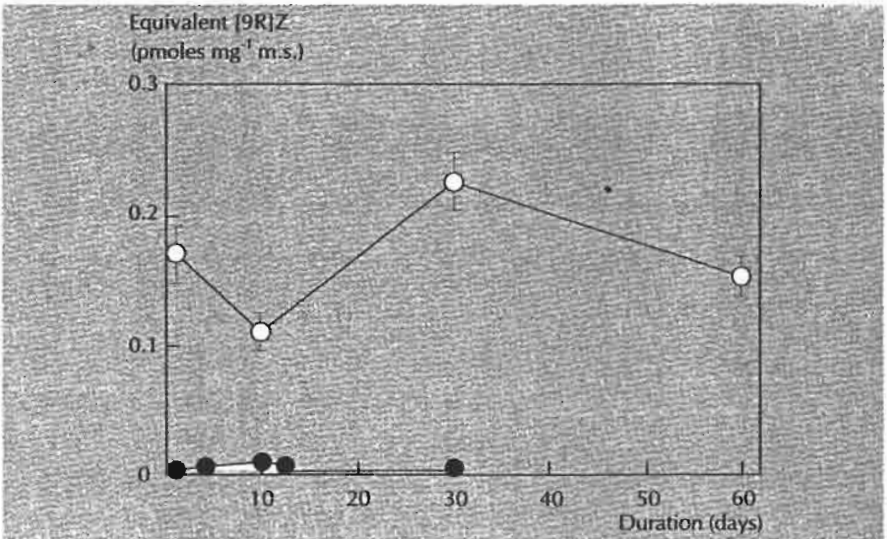


Figure 4. Assessment of levels of [9G]Z in CNC (○) and RGC (●) of the clone C 32 during a culture cycle on standard medium (MI 58).

These values are distribution means (\pm standard deviation).

Bibliography

- BESSE I., VERDEIL J.-L., DUVAL Y., SOTTA A., MALDINEY R., MIGINIAC E., 1992. Oil palm (*Elaeis guineensis* Jacq.) clonal fidelity: endogenous cytokinins and indoleacetic acid in embryogenic callus cultures. *Journal of Experimental Botany*, 43 (252) : 983-989.
- DUVAL Y., DURAND-GASSELIN T., KONAN K., PANNETIER C., 1988. Multiplication végétative du palmier à huile (*Elaeis guineensis* Jacq.) par culture *in vitro* ; stratégies et résultats. *Oléagineux*, 43 (2) : 39-44.
- HANOWER J., PANNETIER C., 1982. In-vitro vegetative propagation of the oil palm, *Elaeis guineensis* Jacq. *In* : *Plant Tissue Culture*, A. Fujiwara, Maruzen eds, Tokyo, p. 745-746.
- MALDINEY R., LEROUX B., SABBAGH I., SOTTA B., SOSSOUNTZOV L., MIGINIAC E., 1986. A biotin-avidin-based enzyme immunoassay to quantify three phytohormones: auxin, abscisic acid and zeatin-riboside. *Journal of Immunological Methods*, 90 : 151-158.
- PANNETIER C., ARTHUIS P., LIEVOUX D., 1981. Néof ormation de jeunes plantes d'*Elaeis guineensis* à partir de cals primaires obtenus sur fragments foliaires cultivés *in vitro*. *Oléagineux*, 36 (3) : 119-121.
- RABECHAULT H., MARTIN J.-P., 1976. Multiplication végétative du palmier à huile (*Elaeis guineensis* Jacq.) à l'aide de cultures de tissus foliaires. *Comptes rendus de l'Académie des sciences, série D*, 283 (16) : 1735-1737.
- SCHWENDIMAN J., PANNETIER C., MICHAUX-FERRIERE N., 1988. Histology of somatic embryogenesis from leaf explants of the oil palm (*Elaeis guineensis* Jacq.), *Annals of Botany*, 62 : 43-52.

Besse I., Beulé T., Verdeil J.L., Duval Yves,
Maldiney R. (collab.), Sotta B. (collab.),
Miginiac E. (collab.) (1994).

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