Journal of Plantation Crops **28** (1): 82-85, April 2000

SHORT SCIENTIFIC REPORTS

VARIATION OF ESTERASE ISOZYME IN REGENERATED PLANTLETS OF OIL PALM (*ELAEIS GUINEENSIS* JACQ)

Manuscript received: 1-4-99; revised: 30-8-99; accepted: 1-11-99.

Oil palm is fast gaining importance as a stable source of edible oil in India. Planting of high yielding palms is being taken up on a large area. Success of this effort requires supply of large number of high quality planting materials. Large scale clonal propagation of suitable palms through tissue culture offers the opportunity to raise quality seedlings in sufficient numbers. Commercial exploitation of this technique demands 'true to type' property of the regenerated plants. Although *in vitro* propagation methods are expected to maintain the characteristics of selected example, variation during tissue culture, commonly known as somaclonal variation (Larkin and Scowcraft, 1987), is a common phenomenon.

Morphological characters, protein, isozymes and molecular markers have been employed to detect somaclonal variation. Although molecular markers like RFLP and RAPD are fast replacing other methods, isozyme analysis is still a method of choice for less equipped laboratories, because of its simplicity and low cost involved. Isozyme markers offer many advantages over morphological markers such as codominant nature, absence of epistatic interactions, small amount of tissue needed and detection at seedling stage itself. We report the presence of variation for esterase isozymes in regenerated plantlets from single oil palm clone.

Emerging shoots from twenty randomly selected regenerated *tenera* plantlets of uniform age was used. These plantlets were regenerated from spindle leaf explant of two year old *tenera* seedling through destructive sampling (Anitha Karun and Sajini, 1996). The shoots were homogenized in 0.1 M sodium phosphate buffer, pH 7.6, in ice. The extract was centrifuged at 20,000 g at 4°C for 15 min. Protein content of the supernatant was estimated as described by Lowry *et al.*, (1951). Aliquots of protein (75-100µg) were loaded on polyacrylamide gel (10% C, 0.1%T). Electrophoresis was carried out with tris-glycine buffer (pH 8.3) at a constant current of 30mA at 4°C. After electrophoresis, gel was incubated for 30-45 min in a solution of 0.15M sodium phosphate buffer (pH 7.2) containing 0.1% Fast Blue RR salt and 0.04% α-naphthyl acetate dissolved in acetone. The gel was destained with water : methanol : acetic acid (15:4:1). The position of the isozyme bands in the gel was expressed as relative mobility (Rf) by measuring the distance migrated by the particular band compared to that of bromophenol blue used as tracking dye during electrophoresis. Rf value was calculated for all the bands and a zymogram showing relative positions of bands in twenty plantlets was constructed. Band frequency was calculated for all the bands. Dissimilarity index was calculated as per the procedure of Nei and Li (1979).

Four major and six minor bands were observed for esterase enzyme (Fig. 1). Major bands (No. 1,2,4,7) were present in all the plantlets except for the absence of band 1 in two plantlets and band 7 in three plantlets. Maximum variation was observed for minor bands. The frequency of bands varied from 0.05 (*Est* 3) to 1.0 (*Est 2* and 4) (Table 1). Based on isozyme pattern these plantlets could be grouped into various classes. Seven plantlets had seven common bands namely *Est* 1, *Est* 2, *Est* 4, *Est* 5, *Est* 6, *Est* 7 and *Est* 8, two had 8 common bands *viz. Est* 1, *Est* 2, *Est* 4, *Est* 5, *Est* 6, *Est* 7, *Est* 9, and *Est* 10, other two plantlets had 6 bands *Est* 1, *Est* 2, *Est* 4, *Est* 5, *Est* 7, *Est* 8, remaining nine plantlets exhibited unique pattern for esterase. The dissimilarity index for these plantlets was 0.6. High dissimilarity index obtained may be due to polymorphic nature of most of the bands (80%).

These results indicate that though regenerated from the same clone, these plantlets may be genetically different somaclones. Various reasons have been proposed for such variations (Larkin and Scowcraft, 1987). Differences in gene expression detected through isozymes are believed to be epigenetic in nature. The epigenetic changes are manifested in the form of morphological characters also, sometimes leading to abnormality in adult palms. The appearance of abnormality in clonal oil palm was first reported by Wooi *et al.*, (1981). They identified abnormal plantlets in the nursery based on their morphology and slow growth. Later several groups reported incidence of abnormalities in the field (Maheran *et al.*, 1995, Durand-Gasselin *et al.*, 1995). Corley *et al.*, (1986) attributed this abnormality to the duration of time for which the tissue have been in culture. While Soh (1987) implied genetic cause

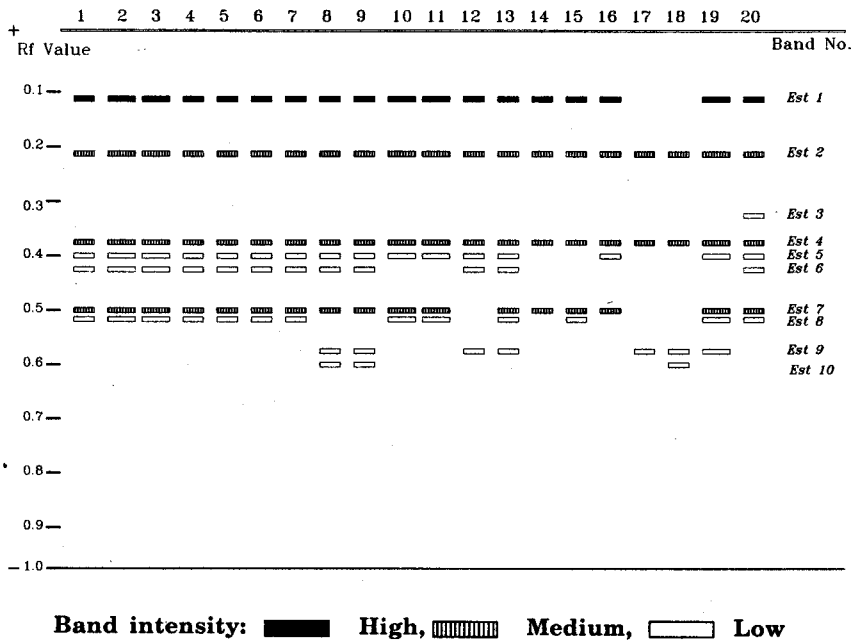

Fig. 1. Zymogram showing esterase bands in 20 tenera regenerated plantlets

Table 1. ***Frequency and nature of esterase bands detected in regenerated plantlets***

Band No.	Frequency	Nature
Est 1	0.90	Polymorphic
Est 2	1.0	Monomorphic
Est 3	0.05	Polymorphic
Est 4	1.0	Monomorphic
Est 5	0.80	Polymorphic
Est 6	0.60	Polymorphic
Est 7	0.85	Polymorphic
Est 8	0.65	Polymorphic
Est 9	0.30	Polymorphic
Est 10	0.15	Polymorphic

for the abnormality, Rao and Donough (1990) also suggested that a genetic information carrier may be implicated in the phenomenon. On the other hand Durand-Gasselin *et al.*, (1995) suggested that disruption in a regulation sequence could be behind this phenomenon. Marmey *et al*,. (1991) detected a protein band specific to fast growing calli (FGC) which regenerate into abnormal plantlet. Paranjothi *et al.*, (1995) observed differences in the profiles of amplified DNA bands of normal and abnormal palms of the same clone. They also found a constituent difference in protein banding pattern of normal and abnormal palms. Tan *et al.*, (1988) detected variation in esterase banding pattern at the tissue culture stage, however, esterase isozyme from leaf tissue of acclimatized plantlets was similar to that in embryogenic cultures. Cheah and Wooi (1995) used molecular markers to analyze DNA changes during subculture *in vitro* and detected one variant at the eleventh subculture, however they attributed it to genetically chimeric nature of callus. While monitoring two clones for clonal fidelity upto 17th subculture, no change in DNA was observed by these workers, suggesting relative stability of the cultures. Similarly genetic stability of oil palm in tissue culture was demonstrated by using cytological techniques (Johes *et al.*, 1982) and field observations of fruit characters (Wooi *et al.*, 1982).

Variation in isozyme pattern specially the absence of major bands in some of the plantlets in this experiment may be indicative of changes in characteristics of the plant (Paranjothi *et al.*, 1995). Such changes may result in abnormal (Marmey *et. al.*, 1991) as well as useful somaclones. Hence isozyme markers may be useful for testing clonal fidelity of regenerates and identification of "off types" or useful materials at an early stage.

REFERENCES

ANITHA KARUN and SAJINI, K.K. 1996. *Curr. Sci.* **71(11)**: 922-926

CHEAH, S.C. and WOOI, K.C. 1995. In: *Proceedings of the 1993 ISOPB International Symposium on Recent Developments in Oil Palm Tissue culture and Biotechnology* (Eds.) Rao, V., Henson, I.E. and Rajanaidu, N., International Society for Oil Palm Breeders, Kuala Lumpur, pp. 163-170.

CORLEY, R.H.V., LEE, C.H., LAW, I.H. and WONG, C.Y. 1986. *The Planter* **62**: 233-240.

DURAND-GASSELIN, J., DUVAL, Y., BAUDOUIN, L., MAHERAN, A.B. KONAN, K. and NOIRET, J.H. 1995. In: *Proceedings of the 1993, ISOPB International Symposium on Recent Developments in Oil Palm Tissue Culture and Biotechnology* (Eds.) Rao, V., Henson, I.E. and Rajanaidu, N.,

International Society for Oil Palm Breeders, Kuala Lumpur, pp. 48-63.

JONES, L., BARFIELD, D., BARFETT, J., FLOCK, A., POLLOCK, K. and ROBINSON, P. 1982. In: *Plant Tissue Culture* (Ed.) Fujiwara, A., Maruzen, Tokyo, Japan, pp. 727-728.

LARKIN, P.J. and SCOWCRAFT, W.R. 1981. *Theor. Appl. Genet.*, **60:** 197-214.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. and RANDALL, R.J. 1951, *J.Biol. Chem.* **193:** 265.

MAHERAN, A.B., AW, K.T., ABUZARIN, O and CHIN, C.W. 1995. In: *Proceedings of the 1993 PORIM International Palm Oil congress 'Update and Vision'* (Eds.) Jalami Sukaimi, Ariffin Darus, Rajanaidu, N., Mohd Iayeb Dolmat, Paranjothy, K,. Mohd. Basri Wahid, Henson, I. E. and Chang, K. C., Palm Oil Research Institute of Malaysia, Kuala Lumpur, pp. 99-113

MARMEY, P., BESSE, I. and VERDEIL, J. 1991. *C. R. Acad. Sci. Paris.* **313** : 333 - 338.

NEI, M. and LI, W. 1979. *Proc. Natl. Acad. Sci. USA* **8**: 5269-5273.

PARANJOTHY, K., ONG, L.M. and SHARIFAH, S. 1995. In: *Proceedings of the 1993 ISOPB International symposium on Recent Developments in Oil Palm Tissue culture and Biotechnology* (Eds.) Rao. V., Henson, I.E. and Rajanaidu, N., International Society for Oil Palm breeders, Kuala Lumpur, pp. 70-85.

RAO, V. and DONOUGH, C.R. 1990. *Elaeis,* **2**: 199-207.

SOH, A.C. 1987. *The Planter,* **63 (731):** 59-65.

TAN, C.S., TARMIZE, A. M. and PARANJOTHY, K. 1988. 7th FAOB symp. Kuala Lumpur. Pos. C-13, 2pp.

WOOI, K.C., WONG, C.Y. and CORLEY, R.H.V. 1981. In: *Tissue culture of economically important plants.* (Ed.) Rao A.N., COSTED and ANBS, pp. 138-144.

WOOI, K.C., WONG, C.Y. and CORLEY, R.H.V. 1982. In : *Plant Tissue Culture* (Ed.) Fujiwara, A., Maruzen, Tokyo, Japan, pp. 749-750.

Biotechnology section,
Central Plantation Crops Research Institute,
Kasaragod - 671 124, Kerala.

ANURADHA UPADHYAY
ANITHA KARUN