Sci. J. Fac. Sci. Menoufia Univ. Vol. IX (1995). 169 - 183

ISOZYME MARKERS ASSOCIATED WITH A PHENOTYPIC MUTANT DERIVED FROM *IN VITRO* CALLUS CULTURE OF *Dieffenbachia pecta var. tropic*

Abou - Ghalia, A. A. and Hamza, H.A.

Genetics Dept. Fac. Agric. Menofiya Univ. Egypt.

ABSTRACT

Peroxidase and esterase isozyme patterns in Dieffenbachia pecta var. tropic and a new leaf form mutant selected from in vitro callus culture were studied. Peroxidases in regenerated mutant showed two extra cathodal bands than in mother plant (control). In contrast, esterases in regenerated mutant showed deficiency in two cathodal bands and only one anodal band. These biochemical characterestics were stable in the five regenerants that have the rounded leaf blade (mutant). It is evident that peroxidase and esterase isozymes could be used as markers for somaclonal variations and early selection.

***Key words** : Somaclonal variations, Isozyme markers, Peroxidase and, Esterase isozymes.*

Introduction

Genetic instability in tissue culture, gives rise for somaclonal variations which can be used for plant improvement by regeneration of mutant plantlets (Carlson and Polacco, 1975, Green, 1977). Larkin and Scowcroft (1981), reported that many phenotypic variations appeared among the regenerated plantlets during tissue culturing. Qureshi *et al.* (1992) reported looseness of the organizational constraints of tissues that allowed plant cells to grow in an unorganized manner. Apparently, this removal of organizational

coherence result in genomic instability which often leads to chromosomal aberrations (Karp and Maddock, 1984, Chen *et al.*, 1987), mutations (Evan and Sharp 1983), activation of transposable elements, and gene amplification (Larkin 1985). The variability of tissue culture - derived progeny arising from culture-induced genomic instability is termed somaclonal variation (Larkin and Scowcroft, 1981). In the literature, it has been reported that the use of some regulators such as 2,4-D and 6-BA lead to an increase in the number of somatic mutations (D'Amato 1977). Sasagaw and Matsushima (1991), reported that IAA and its derivatives are not mutagenic in *Salmonella typhimurium* and *Escherichia coli*.

Electrophoretic zymograms are stable varietal charactaristic and could be used in varietal identification (Gorman and Kiang 1977). Oono *et al.* (1978) used esterase isozymes as markers for *in vitro* rice studies and found some variations of activities during callus induction and regeneration. Romero *et al.* (1993) reported that isozyme analysis provides a definitive evidence for the degree of homology between rice species. Sun *et al.*, (1977) showed that albino mutants regenerated from rice callus culture were charactarized by the lacking of one band of soluble proteins. Abou-Ghalia (1990) showed that peroxidase isozymes can be used as markers for salt-tolerance plantlets selected in tomato derived from *in vitro* callus culture. Mangoline *et al.* (1994) found that, esterase, peroxidase and acid phosphatase could be used as markers of *Cereus preuvianus* plants regenerated from callus culture.

In this study, *Dieffenbachia pecta* var. tropic was manipulated by tissue culture. A new leaf shape mutant (rounded leaf blade) was

selected as a somaclonal variant. The mutant plantlets (five plantlets) were adapted to peat medium and grown in green house for two months. Isozymes zymograms of peroxidases and esterases were studied. Variations were obtained for both peroxidases and esterases when comparison was made between the mother plant and the mutant. The hypothesis that search for isozyme band (s) alterations could be used as a marker for early detection of phenotypic variants resulting from *in vitro* callus culture in *Dieffenbachia* was discussed.

MATERIALS AND METHODS

1. Mutant plantlet isolation :

Five mutant plantlets were selected from 2500 plantlets obtained during tissue culturing on MS medium (Murashige and Skooge, 1962) supplemented with IAA (0.2 mg/l.) and 6-BA (1.0 mg/l) for callus induction stage. Regeneration of shoots was on MS medium containing IAA (0.1 mg/l.) and 6-BA (6.0 mg/l.) while, root formation was on MS medium supplemented with IAA (o.2 mg) and 6-BA (2 mg/l.). Selected mutant was adapted in peat medium for two months in green house.

2 - Plant material preparation for electrophoresis :

Plantlets were homogenized in cool mortar (0.5 gm fresh weight). the homogenate was diluted with 0.5 ml d.H_2O and centrifuged at 1000 rpm for 3 min. under cooling.

3 - Gel buffers for peroxidase and esterase :

i - Electrode buffer

O.3M boric acid - sod. hydroxide buffer pH 8.0 were prepared (El-Metainy *et al.* 1977 a).

ii - Gel buffer

0.03 M boric acid - sod. hydroxide buffer pH 8.0 .

4 - Gel media for peroxidase and esterase :

Agar - pvp gel : 1.0gm Agar and 0.5 gm pvp (MW 10,000) were added to 100 ml of gel buffer. the mixture was cooked in boiling water bath until the solution became transparent. Horozintal gel trays were prepared by pouring the solution on a glass plate 20 x 27 cm., and kept at 4°C until utilization (El-Metainy *et al.*, 1977 b).

5 - Staining Solutions :

i - Peroxidase staining solution :

0.1 gm benzidine dissolved in 1.0 ml acetone, then 98.0 ml of 0.01 M Sodium acetate - acetic acid buffer pH 4.7 were added. 1.0 ml of H_2O_2 (10 vol.) was added immediately before staining.

ii- Esterase staining solution :

Sol. a :

50 mg of α - Naphthyle acetate and 55mg of β - Naphthyle acetate were dissolved in 20 ml acetone.

Sol. b:

Fast blue - RR (50 mg) and Fast blue - BB (50 mg) were

dissolved in 80 ml d. H_2O. Solutions a and b were mixed immediately before use.

6 - Procedure :

Supernatent of the homogenate plantlet was absorbed on 1.0 x 0.2 cm strips of Whatman No. 1 filter paper. Strips were placed on the surface of Agar plate for 15 min at 4°C, then strips were removed. After 25 mA. for one plate for 3 hrs at 4°C using 0.3M boric acid - NaoH buffer pH 8.0 as electrode buffer, the gel plates were stained with specific staining sloution.

RESULTS AND DISCUSSION

The phenotypic mutant of *Dieffenbachia pecta var. tropic* isolated from *in vitro* callus culture (Fig. 1) indicated that, the tissue culture process gave rise to somaclonal variation. Studies showed that the differences between the mutant (rounded leaf blade) plants and the mother plant (narrow leaf blade) were clear at two levels, phenotypically and biochemically. The phenotype of the mutant is different from the mother plant in leaf form and variegation pattern The leaf's blade is round in mutant and narrow in control. In tomato a potato leaf form mutant was found (Rick 1956), it was a recessive single gene controled character. Evan and sharp (1983) found a single gene mutation in tomato plants regenerated from tissue culture. In *Diffenbachia* variegation was found to be a sinlge dominant allele (Henny 1986).

Electrophoretic zymogram of peroxidase (Fig. 2 A and B) in mother plant (control) showed 7 peroxidase isozyme bands, two of

Fig. (1) : Rounded leaf blade mutant (A) and Narrow leaf blate as control (B) of *D. Pecta var. tropic.*

A	B
A New leaf form (rounded blade derived from *in vitro* cullus culture of *D. pecta*	contol (Narrow blade)

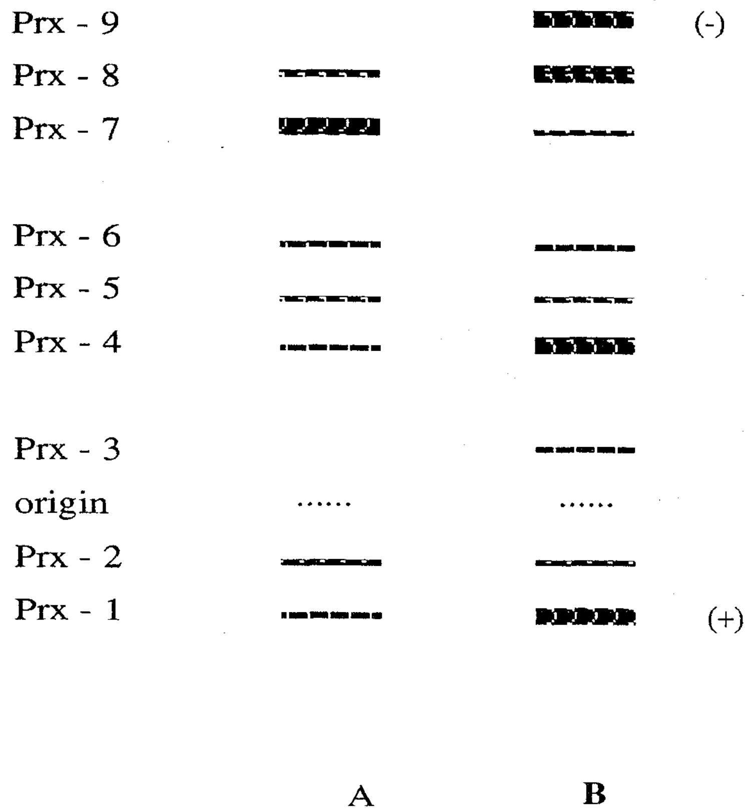


Fig. (2) Peroxidase isozymes zymogram of *D. Pecta* B(control and C(rounded leaf blade mutant).

them migrated to anodal front while, the other 5 bands migrated toward cathode. The mutant plants showed two extra cathodal bands. The two extra bands were the smallest bands in weight while the third one had the largest weight in the cathodal front (Fig. 3). Also, mutant showed high activity for prx-8 and prx-9 while it showed low level of activity for prx.-7. On the other hand, the zymogram of peroxidase for mother plant showed high activity in prx-7 while, prx-8 showed low activity. These variations in peroxidase activities reflect variations in peroxidase gene (s) expression. Variation in gene expression can be due to mutation in peroxidase gene (s). The activity of peroxidases estimated visually. these results are in agreement with Fuki's (1983) results in albino rice plantlets.

Esterases zymogram (Fig. 4 A) of the mother plant showed 8 bands, three of them migrated toward anode while the other 5 bands were cathodal bands. (Fig. 5) Regenerated plantlets which have normal phenotype showed abcense of only one cathodal band (Fig. 4 B) termed Est-7 on the other hand, the mutant plantlet zymogram was lacking in two cathodal bands (Est-7 and Est -8) and only one anodal band (Est-1) when compared with control (Fig. 4 C). Variation in Esterases activity was estimated visually. As shown in zymograms (Fig. 4 A, B, and C), the mother plant showed high activity in Est-7 and Est-8 and low activity in Est-6 (Fig. 4 A). However, normal regenerated plants showed low activity in Est-8 and some of them showed high activity for Est-6 (Fig. 4 B). Zymogram of mutant plant (Fig. 4 C) indicate that Est-6 and Est-2 have high activity when compared with control. These results were supported by Oono *et al.* (1978) results in rice. Colourimetric studies must be done to confirm

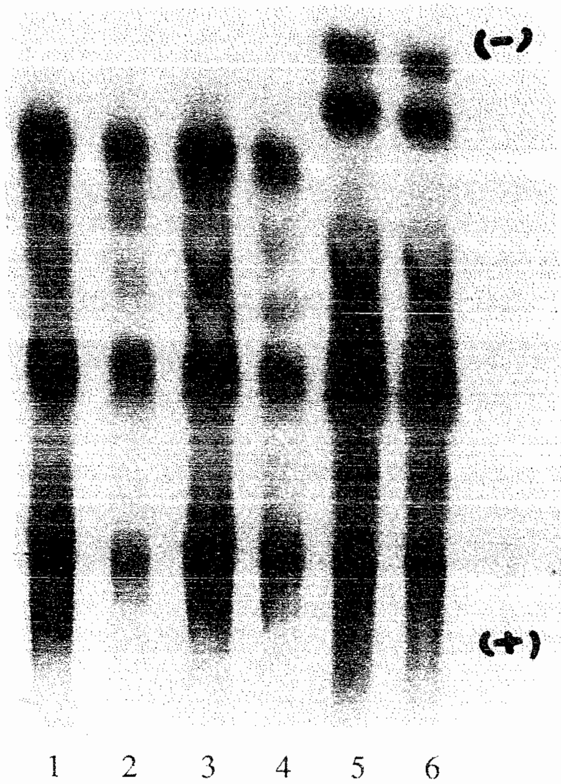


Fig. (3) : Perxidase isozymes variation Lane 1, 2, 3, and 4 for control. lanes 5 and 6 for the mutant.

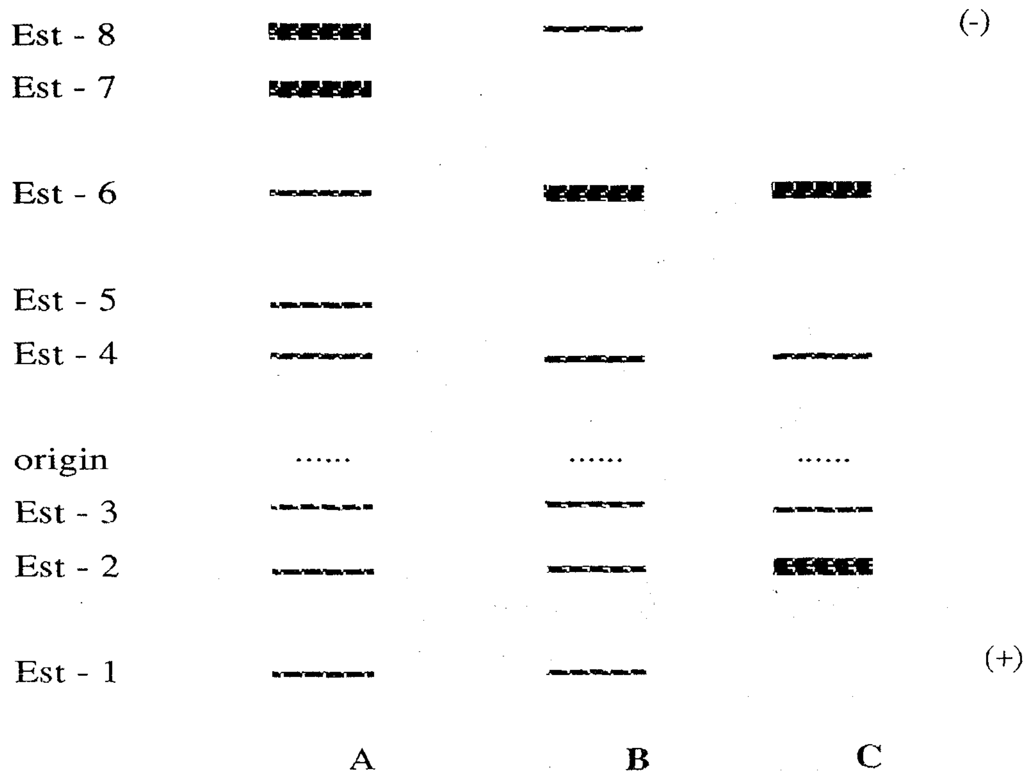
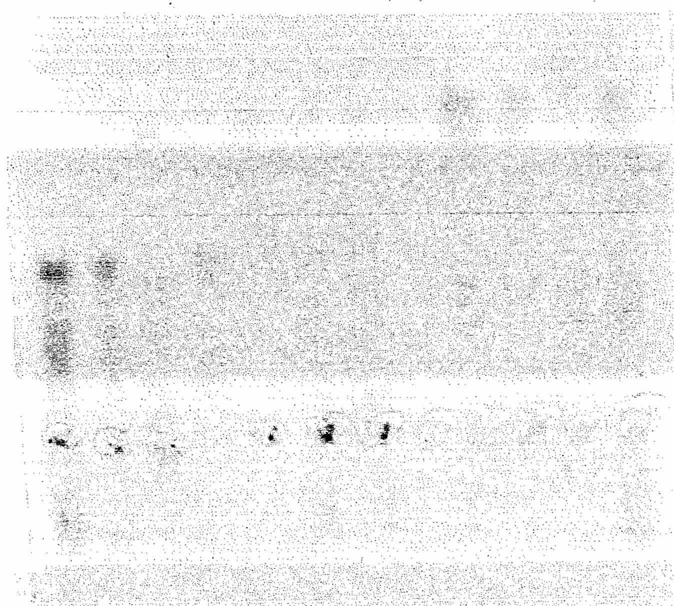


Fig. (4) Esterase zymogram in *D. pecta var. tropic* (A) normal regenerants (B) and rounded leaf blade mutant (C).



1 2 3 4 5 7 8 9 10 11 12

Fig. (5) : Esterase isozymes variations in *D. pecta var. tropic* (lanes 9, 10, 11 & 12), normal regenerants (lanes 3, 5, 6, 7 & 8) and rounded leaf blade mutants (lanes 1, 2 & 4).

the variation in peroxidase and esterase activities.Qualitative variations in both peroxidase and esterase zymogram can be used as good markers for phenotypic variations and can be used as markers for early selection for somaclonal variants induced by *in vitro* culture of *Dieffenbachia*. According to D'Amato (1977 and 1978) and Matsushima (1991) results, we could expect that the selected mutant of *Dieffenbachia* leaf form may be induced by 6-BA during tissue culture course. These results confirmed that the morphological changes are accompanied with biochemical changes.

REFERENCES :

Abou - Ghalia, A.A. (1990) : Genetic studies on *Lycopersicon spp*. ph.D. thesis. Genetics Dpt. Fac. Agri. Menofiya Univ. PP. 114 - 117.

Carlson, P.S. and Polacco, J.C. (1975) : Plant cell cultures Genetic aspects of plant improvement, Science (Washington. D.C.) 188 : 622 - 625.

Chen, T.H.H., M.D. Lazar, G.J. Scoles, L.V. Gusta an K.K. Kartha (1987) : Somaclonal variation in a population of winter wheat. J. Plant Physiol. 130 : 27 - 36.

D'Amato, F. (1977) : Cytogenetics of differentiation in tissue and cell culture. In : Applied and fundamental aspects of plant cell, tissue and organ culture (Reinert, J. and Bajaj, Y.P.S. eds.); pp. 343 - 357. Berlin, Heidelberg, New York : Springer.

D'Amato, F. (1978) : Chromosome number variation in cultured cells and regenerated plants. In : Frontiers of plant tissue culture (Thorpe, T. A. ed.), PP 287 - 295. Intern. Ass. plant tissue culture, Univ. of Calgary Offset printing services, Calgary, Canada.

El-Metainy, A.Y.; Abou - youssef, A.Y.; Sherif, M.I. and Sahrigy, M.A. (1977 a) : Isozyme variations in *Lycopersicon species*. Egypt. J. Genet. Cytol. 6 ; 360 - 369.

El-Metainy, A.y.; Abo - youssef, A.y. and El-Hadad, M. (1977 b) : Starch degrading isozymes in *Triticun aestivum, T. pyramidal* and their interspecific hybrid. Egypt. J. Genet. Cytol. 6 375 - 379.

Evans, S.A. and W.R. Sharp (1983) : Single gene mutations in tomato plants regenerated from tissue culture. Science 227 : 949 - 957.

Fuki, K. (1983) : Sequential occurrence of mutation in growing rice callus. Theor. Appl. Genet. 65 : 22 - 230.

Gorman, M.B. and Kiang, Y.T. (1977) : Variety specific electrophoretic variants of four soybean enzymes. Crop Sci. 17 : 963 - 965.

Green, C.E. (1977) : Prospect for crop improvement in the field of cell culture. Hort Sci. 12 : 131 - 134.

Henny, R.J. (1988) : Inheritance of foliar variegation in *Dieffenbachia maculate* "Camill". J. Heridity, 77 : 4; 285 - 286.

Karp. A. and S.E. Maddock (1984) : Chromosome variation in wheat plants regenerated from cultured immature embryos. Theor. Appl. Genet. 67: 249 - 255.

Larkin, P.J. (1985) : *In vitro* culture and cereal breeding. In. S.W.J. Bright and M.G.K. Jones (eds.). Cereal tissue and cell culture. Martinus Nijhoff, Dr. W. Junk publishers, Dordrecht, Boston, Lancaster. PP. 274 - 296.

Larkin, P.J. and Scowcroft W.R. (1981) : Somaclonal variatiion. A novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60 : 197 - 214.

Mangolin, C.A.; Prioli, A.J. and Machodo. M.F. (1994) Isozyme patterns in callus cultures and in plants regenerated from calli of *Cereous Peruvianus* (Cactaceae). Biochem - Genet. 32 (7 - 8) 237 - 247.

Murashige, T.A. and Skooge, F. (1962) : A revised medium for rapid growth and bioassay with tobacco cultures. Physiol. plant, 15 :473 - 497.

Oono, K.; Nakagohar, M. and Hayashi, (1978) : In "Studies of single cell culture on higher plants" (Watanabe, Y. ed.) Series 109, P 58. Research council, Minist, Agric. Forest. Tokyo.

Qureshi, J.A., K.K. Karthia, S.R. Abrams and *L. steinhaauer*, 1989. Modulation of somatic embryogenesis in early and late stage embryos of wheat (*Triticum aestivum* L.) under the influence of (± L. abcisic aad and its analogs. Plant cell tissue organ culture. 18: 55 - 69.

Rick, C.M. (1956) : "Cytogenetics of the Tomato" Advances genetics. Vol. VIII P. 280 (Demerec. ed.).

Romero, G.O. Amante - Bordeos, D.A.; Dalmacis, D.; Eloran R. and Sitch, A.L. (1993) : Comparitive studies of isozymes in *Oryza sativa, O. minuta,* and their interspecific derivatives : evidence for homology and recombination. Theor. Appl. Genet. 87 : 609 - 615.

Sasagaw, and Matsushima, (1991) : Mutagen formation on nitrate treatment of indole compounds derived from Indole - glucosinolate.Mutat. Res. 250 (1-2) : 169 - 74.

Sun, C.S. Wu, and Wang, C. (1977) : In : Proceeding of symposium on plant tissue culture. P. 151 Science press. Peking.

ملخص عربى

المرقمات الانزيمية المرتبطة بطفرة مظهرية ناتجة من الزراعة النسيجية فى نبات الديفينباخيا

عاطف ابوغالية ، حنفى حمزه

تم دراسة المشابهات الانزيمية لانزيم البيروكسيديز والاستيريز فى نباتات الديفنباخيا وطفره منتخبة من بين التباينات النسيجية الناتجة عن زراعة الأنسجة .

أظهرت الدراسة وجود مشابهين أنزيميين للبيروكسيديز جديدين يهاجران ناحية القطب السالب فى الطفره المنتخبة بينما أظهرت الدراسة غياب ثلاث مشابهات أنزيميه للاستيريز أثنان منها يهاجران للقطب السالب والثالث يهاجر للقطب الموجب .

هذه الخواص الكيموحيوية للطفره المورفولوجية فى الديفنباخيا (ورقه مستديره) كانت ثابته فى الخمس نباتات المنتخبة كطفرة من بين التباينات النسيجية .

من الدراسة السابقة يمكن استنتاج أن الاختلافات فى المشابهات الانزيمية لكل من البيروكسيديز والاستيريز يمكن استخدامها كمرقمات للتباينات النسيجية الوراثية ويمكن استخدامها فى الانتخاب المبكر للاختلافات المورفولوجية التى قد تنتج عن الزراعة النسيجية