ESTERASE ISOZYME PATTERNS IN RICE SOMATIC ORGANS AND THE 2, 4-D INDUCED CALLUS TISSUES[1]

LIN WU and H. W. LI[2]

(Accepted Aug. 10, 1970)

Abstract

Esterase isozymes in different somatic organs of rice and the 2.4-D induced callus tissues were separated with acryamide-gel-electrophoresis. Using α-naphthylacetate as substrate, altogether seven bands of esterase isozymes were identifiable in the electrophoretograms. Eight bands could be detected if β-naphthylacetate was used as subtrate. Number as well as concentration on the esterase bands vary with different somatic organs. However, callus tissues induced from different somatic organs exhibited uniform patterns of zymogram.

Introduction

Introduction of improved methods for the gell electrophoretic separation of different molecular forms of enzymes, coupling with various enzyme staining techniques (Hunter and Markert 1957, Markert and Miller 1959, Whipple 1964, Thueman, Plain and Laycock 1965), have encouraged numerous isozyme studies in higher plants (Scandalious 1964, Eavans and Alldridge 1965, Vaughan 1966, Schwartz 1967, Van Loon and Van Kammen 1968, Robert 1968, Williamson and Kleese 1969).

This investigation compares the esterase isozyme patterns of different somatic organs and the callus tissues induced by 2.4-dichlorophenoxy acetic acid (2.4-D).

Materials and Methods

Somatic organs used in this study were removed fresh from *Oryza sativa*, Taichung No. 65. The callus tissues were induced originally from roots, coleoptiles, nodes, cotyledonous nodes and scutella (Wu and Li 1970) and re-

1 Supported in part by the National Science Council of the Republic of China. Scientific Paper No. 105, Institute of Botany, Academia Sinica.

2 Assistant Research Fellow and Research Fellow respectively Institute of Botany, Academia Sinica.

spectively subcultured on agar medium (Maeda, 1967) supplemented with kinetin 2 ppm, indoacetic acid 2 ppm and 2.4-D 4 ppm. The cultures were maintained in darkness at 28°C for sixty days before used.

For isozyme analysis in higher organisms various solvents with different pH values have been employed to extract the enzymes. For example, distilled water (Boulter, Thurman and Turner 1966, Zen-ichi Ogita 1964), 0.1 M tris-buffer at pH 7.0 (Daniel, Menzel, Roderick and Hoslins 1963, Bienvernido, Juliano and Vanner 1969), 0.1 M potassium sulphate buffer at pH 7·2 (Thurman, Palin and Laycock 1965) and 0.1 M Tris- borate-EDTA buffer at pH 8.9 (Ma and Li 1969) were used by many workers. In order to obtain maximum separation of esterase isozymes, the aforementioned solvent systems were screened in our preliminary experinments. Since maximum number of esterase bands was obtained using distilled water or 0.1 M potassium sulphate buffer at pH 7.2 in our experiment, distilled water was chosen as the extraction solvent. Three grams of the fresh tissues was homogenized in 1 ml of distiled water with a mortar. The homogenats were than centrifuged at 20,000×g for 20 min. The supernatants were immediately used or stored in a deep-freezer untill needed.

Gel preparation:

Five per cent acrylamide gel was prepared according to Nernberg (1966), except glycine tris-buffer was replaced with 0.1 M tris-borate EDTA buffer at pH 8.9. Each glass tube (6.5×0.65 cm internal diameter) was filled with poly-acrylamide gel up to 6 cm hight.

Electrophoresis:

The acrylamide gel disc-electrophoresis was performed in 0.1 M tris-borate EDTA buffer system at pH 8.9. 50 μl of the sample was applied on the top of each gel column with a hypodermic syringe. A D. C. current of 5 mA per tube was applied for one hour. The gels were then removed from the tubes by rimming around the glass wall with a syring needle.

Gel staining:

Gels removed from the columns were incubated in 0.5 M boric acid in cold room (5°C) for 1.5 hrs. Staining was performed in 0.1 M Na-phosphate buffer at pH 6.5 containing 0.5 mg/ml Fast red salt TRN and 4 mM of either α-naphthylacetate or β-naphthylacetate (dissolved in 2 ml of 50% acetone) as substrates. Staining was terminated after overnight incubation in cold room.

Results

The crude enzyme extracts were placed respectively at anode or cathode. However, no migration of esterase isozyme toward cathode was detected

throughout this investigation. This part of the electrophoretograms, therefore, are not presented here.

Intensities of the esterase bands in zymograms are indicative of the enzyme concentrations. The bands therefore are artificially grouped in to three categories according to the intensities as shown in schematic zymograms (Fig. a, b, c, and d).

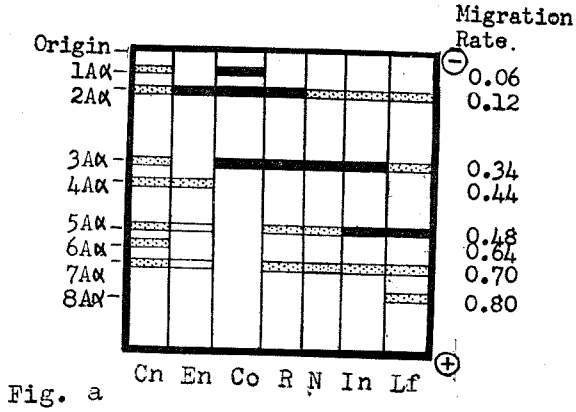

Fig. a

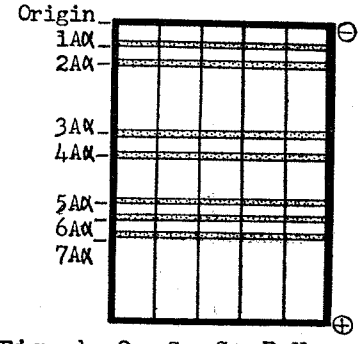

Fig. b

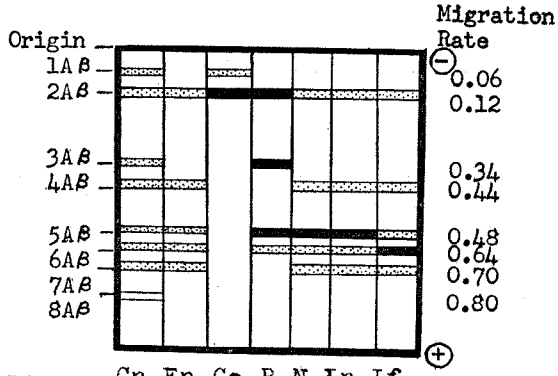

Fig. c

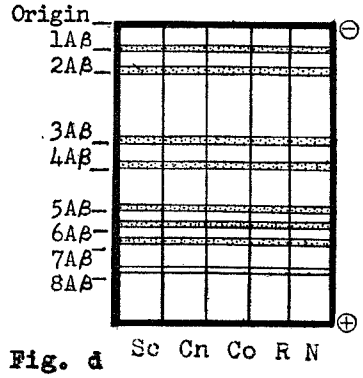

Fig. d

Schematic zymographs of α-(Fig. a) and β-naphthylacetate (Fig. b) specific esterase isozyme patterns of different somatic organs and callus tissues (Fig. c and d) of *Oryza sativa* Taichung No. 65; embryo (En), coleoptile (Co), root (R), node (N), inter node (In)' scutellum (Sc).

Using α-naphthylacetate as substrate, different esterase zymogram patterns were detected from different somatic organs (Fig. a). Accrording the positions in the electrophoretic fields, seven distinct bands can be recognized for the esterase isozyme from the somatic organs investigated.

However, regardless of the types of somatic tissues from which they were induced, callus tissues exhibited identical esterase zymogram patterns (Fig. c).

Figs. b and d are the esterase zymograms using β-naphthylacetate as substrate. Different somatic organs again showed different isozyme patterns whereas the callus tissues from various sources are indistinguishable from one another as far as the zymogram patterns are concerned (Fig. c and d).

Discussion

This study confirmed that esterase existed in multiple forms in rice plant reported by Ma & Li (1969). Altogether seven migrating bands of α-naphthylacetate specific esterase were detected from somatic organs. These bands were overlaped by those of β-naphthylacetate specific esterase, except the bands 8Aα and 6Aα.

The isozyme bands detected from callus tissues included all the bands from different somatic organs except the band 8Aα which was found only from leaf-blade. It is suggested that the synthetic system of 8Aα is controled by certain mechanism which is not affected by 2.4-D. Two bands, 6Aα and 8Aβ, however, from callus tissues were not found in any of the six organs. It is postulated that they may exist in some other organs not examined in this study.

The uniformity of isozyme patterns in rice callus tissues indicated that 2.4-D may in some ways inactivate the regulatory mechanisms which in normal somatic organs suppress the biosynthesis of certain molecular species.

用膠凝體電泳法 (acrylamide-gel-electrophoresis) 對水稻體不同器官，與以 2.4-D 誘發所得腫瘤組織內酯化酵素 (esterase) 之分析

吳 麟 李先聞

用膠凝體電泳法 (acrylamide-gel-electroyhoresis) 分析水稻六個不同器官，以及自五個不同器官以 2.4-D 誘發出之腫瘤組織 (callus tissue) 中的脂化酵素 (esterase)。以 α-naphthylacetate 做爲反應物質 (substrate) 共分析得七個同位酵素 (isozyme)，若以 β-naphthylacetate 做爲反應物則呈現八個同位酵素。其同位酵素的數目與濃度均隨器官之不同而有改變，但分析五種來源不同的腫瘤組織其同位酵素的數目均相同，而各同位酵素間之濃度亦無明顯的差異。

Literature Cited

BIENVERNIDO O. JULIDO and J. E. VARNER 1965. Enzymic Degradation of Starch Granules in the Cotyledins of Germinating Peas. Plant Physiology. Vol. 44, No. 6, 886-892.

BOULTER, D. A. THURMAN and B. L. TURNER 1966. The Use of Disc Electrophoresis of Plant Proteins in systematics. Taxon **15** (4): 135-143.

D. A. THURMAN, C. PALIN, M. V. LAYCOCK. 1665. Nature Vol. 207, No. 4993, 193-194.

DANIEL B. MENZEL, RODERICK CRAIG and W. M. HOSKINS. 1963. Electrophoretic properitics of esterase from susceptible and resistant strains of the housfly Musca Domestica L. Ins. Physiol. Vol. 9, 479-493.

EAVANS J. J. and N. A. ALLDRIDGE. 1965. The distribution of peroxidases in extereme drawf and normal tomato. Phytochem. 4: 499-503.

HUNTER R. L. and C. L. MARKERT. 1957. Histochemical demonstration of enzymes separated by zone electrophoresis in starch gels Science **125**: 1294-1295.

MAEDA. 1965 Callus formation and Isolation of single cells from rice seedlings. Proceeding of the Science Socity of Japan Vol. XXXIV No. 2 139-148.

MA Y, and H. W. LI. 1969. Studies on the polyarymide-gel electrophoresis of rice isozymes. Bot. Bull. of Aca. Sin. Vol. X. No. 1 29-35.

MARKERT, C. L. and F. MOLLER. Multiple forms of enzymes: Tissue ontogeny and species specific patterns. Proc. Natl. Acad. Sci. **45**: 753-763.

NERNBERG S. T. 1966. Electrophoresis: A. practical laboratory manual. F. A. Davis Company.

SCANDALIOUS J. G. 1964. Tissue-specific isozyme variation in maize J. Heredity **55**: 281-285.

SCHANNON L. M. 1667. Plant isozymes Ann. Rev. Plant physiol. **19**: 187-210

SCHAWARTZ D. E. Esterase isozymes of maize: On the nature of the gene-controlled variation. Proc. N. A. S. **58**: 568-575

THURMAN, D. A., C. PALIN and M. V. LAYCOCK, 1965. Isoenzymatic nature of L-Glutamic Dehydrogenase of higher plants. Nature 207, No. 4993 193-194.

VAN LOON L. C. and VAN KAMMEN. 1968. Polyacrylamide disc electrophoresis of the soluble leaf proteins from Nicotiana Tabacum Var "Samsun" and "Samsun NN-1" Phytochem. **7**: 1727-1735.

VAUGHAN, J. G. 1966. Comparative electrophoretic of the seed proteins of certain species of Brassica and Sinapis J. Exper. Bot **18**: 100-109.

WU L. and H. W. Li. 1970. Induction of callus tissues Initiation from different somatic organs of rice plant by various concentration of 2.4-dichlorophonexy acetic acid. Cytology in press.

WILLIAMSON, J. A. and J. R. KEESE, 1969. Electrophoretic variation in esterase of three variations of Oats. Nature. **220**: 1134-1136.

WHIPPLE, H. E. 1964-Gel electrophoresis (A conference) Ann. N. Y. Acad. Sci. **121**: 305-650.