Kasetsart J. (Nat. Sci. Suppl.) Vol. 27, : 43-51 (1993)

Isozymes Electrophoresis for Varietal Verification in Some Tropical Vegetables

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ABSTRACT

Techniques for varietal identification of vegetable crops using seed protein and isozymes electrophoresis were investigated. Several isozymes namely, alcohol dehydrogenase (ADH), peroxidase, esterase, glutamic oxaloacetic transaminase (GOT), phosphoglucomutase (PGM) and malate dehydrogenase (MDH) were analysed to standardize in proper condition of plant ages, plant parts, sample extraction solution, gel concentration and staining solution. It had been found that 12 % gel was suitable to analyse isozymes and 13.5 % for protein. Isozymes extracted with phosphate buffer, pH 7.5-8.5 from leaves of 7 day old seedling showed the best expression of banding pattern and distinguish the difference among breeding lines, namely peroxidase in sweet corn, ADH, PGM and esterase in Brassica; esterase in tomato and okra

Keywords: electrophoresis, identification, isozymes

INTRODUCTION

Plant varietal identification is a critical activity in breeding programs to control propagation and quality of products. Phenotypic diescription in the field is time- and labor-consuming and often provides ambiguous descriptors. Isozymes and protein electrophoresis have been developed and recently used for genotypic description (Gupta and Robbelen, 1986; Draper and Cooke, 1987; and Glazmann, 1988). We attempted to establish techniques for routine work on application of isozymes and protein to examine the varietal verification in some vegetable crops.

MATERIALS AND METHODS

Isozymes were extracted from leaf , shoot and root of 5 day-or 7 day-seedling of okra, tomato, sweet corn and Brassica. The extractions were processed under cold condition using mortar with either 0.1 M phosphate buffer, pH 7.5 or 0.05 M Tris buffer, pH 8.5 for 1 ml. After centrifuging, supernatant of extracted solution was taken and added with 0.1 ml glycerol. The extracted isozymes solution could continue loading in electrophoresis gel or keep under -20°C. Protein was extracted by Tris-buffer, pH 8.5 and 2-mercaptoethanol.

The isozymes was loaded in polyacrylamide gel of seperating and stacking gel of Tris buffer pH 8.3. Protein was loaded in 13.5 % polyacrylamide gel of 1 m Tris. HCL pH 8.8 and 0.27% SDS for seperating and 0.25 M Tris.HCL pH 6.8 and 0.2 % SDS for stacking gel. Electrophoresis buffer of protein comprised of 3.0 g Tris, 14.4 g glycine, 1.0 g SDS in 1000 ml distilleted water.

Staining solution for several isozymes namely, alcohol dehydrogenase (ADH), peroxidase, esterase, glutamic oxaloacetic transaminase (GOT), phosphoglucomutase (PGM) and malate dehydrogenase (MDH) were proceed as shows in Table 1

RESULTS AND DISCUSSION

The several isozymes namely, alcohol dehydrogenase (ADH), peroxidase, esterase, glutamic

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Table 1 Staining solution of protein and isozyme.

Protein, isozymes staining	Chemical names	mg, ml/1,000ml dist. water
Total Protein	**Coomasie Brilliant Blue R250**	**1 g**
	Methanol	**500 mL**
	Acetic acid	**100 mL**
Esterase	A. Phosphate Buffer 0.1 M pH 6.0	100 ml.
	Fast blue B salt	150 ml.
	B. 1% α-Napthy. acetate + 1% β-Napthyl acetate (in alcohol absolute)	3 ml.
Peroxidase	A. 3 - amino - 9 - ethylcarbazol	420 mg.
	β - Napthol	290 mg.
	Acetone	200 mg.
	B. Tris - buffer 0.0125 M pH 4.0	
	- Tris hydroxymethyl aminomethane	3.78 g.
	- Acetic acid	4.05 ml.
	- H_2O to	2.5 l.
	C. H_2O_2 3%	
	- H_2O_2 30%	10 ml.
	- H_2O_2 to	100 ml.
	Mixing of A,B and C in ratio 20:8:1 by volume	
Glutamic Oxaloacetic Transaminase		
	A. Phosphate Buffer 0.1 M pH 8.0 (or Tris Buffer 0.1 M pH 8.5)	100 ml.
	α- Ketoglutaric acid	100 mg.
	L- Aspartic acid	200 mg.
	B. Pyridoxal - 5 - phosphate	10 mg.
	Fast Blue BB salt	200 mg.
Malate Dehydrogenase		
	Tris buffer pH 8.5 (0.5 M)	20 ml.
	Sodium - DL - malate	20 ml.
	NAD +	0.02 g.
	NBT (50 mg/ H_2O 5 ml)	1.0 ml.
	PMS (5 mg/ H_2O 5 ml)	0.1 ml.
	H_2O	60 ml.
Acid phosphatase		
	A. Fast Garnet GBC Salt or Fast blue RR salt	150-200 mg.
	B. α + B Napthyl acid phosphate solution	3-4 ml.
	C. Acetate buffer 0.5 M pH 4.8	100 ml.
	D. $MgCl_2$ - $6H_2O$ 1M	1 ml.
	α-β Napthyl acid phosphate	
	- 50% Acetone	10 ml.
	- α-Napthyl acid phosphate	100 mg.
	- β-Napthyl acid phosphate	100 mg.

Table 2 Appropriate condition for preparing plant and isozymes sample of different kind of vegetables for polyacrylamide gel electrophoresis and its staining.

Okra

Isozymes Extraction buffer	Plant age	Plant part	Concentration of gel		Staining
			Seperating gel	stacking gel	
Esterase					
Phosphate buffer 0.05 M pH 7.5 1 ml + PVP 5%	2, 5, 7, 10*	1 dry seed 2 seedling - root - stem + cotyledon leaf	12.5 %	4.5 %	incubate in the dark at 40°C at for 60 min
Tris-buffer 0.05 M pH 8.5		3 cotyledon* leaf 4 leaf			
Peroxidase					
Phosphate buffer* 0.05 M pH 7.5 1 ml + PVP 5%	2, 5, 7, 10*	1 dry seed 2 seedling - root - stem + cotyledon leaf	12.5%	4.5 %	incubate in the dark at 30°C at for 30-60 min or until band appear
Tris-buffer 0.05 M pH 8.5		3 cotyledon* leaf 4 leaf			
Acid phosphatase					
Phosphate buffer* 0.05 M pH 7.5 1 ml. + PVP 5%	2, 5, 7, 10*	1 dry seed 2 seedling - root - stem + cotyledon leaf	12.5 %	4.5 %	incubate in the dark at 30°C at for 30-60 min
Tris-buffer 0.05 M pH 8.5		3 cotyledon* leaf 4 leaf			
Esterase - 0.05 M phosphate buffer* pH 7.5 - PVP 5% + Triton-x 0.05% 1 ml.	5,7*,13	leaf	12.5%	4.5% 40°C for	incubate in the dark at 30-60min
peroxidase - 0.05 M phosphate buffer* pH 7.5 - PVP 5% Triton-x 0.05% 1 ml	5,7* ,13	leaf	13.5%	4.5%	incubate in the dark at 30°C for 30 min

Table 2 Appropriate condition for preparing plant and isozymes sample of different kind of vegetables for polyacrylamide gel electrophoresis and its staining.(continued)

Okra

Isozymes	Extraction buffer	Plant age	Plant part	Concentration of gel		Staining
				Seperating gel	stacking gel	
GOT	- 0.05 M phosphate buffer* pH 7.5 - PVP 5% + Triton-x 0.05% 1 ml.	5,7*,13	leaf	13.5%	4.5%	incubate in the dark at 30°C for 30-60min or until band appear
Malate dehydrogenase	- 0.05 M phoshate buffer pH 7.5 - PVP 5% Triton-x 0.05% 1 ml	5, 7, 13	leaf	7.5%	3.75%	incubate in the dark at 30°C for 15-30min or until band appear
Protein	- Tris -HCl pH 8.0 500 µl + 10 µl 2-mercaptoethanol	7	leaf	13.5%	4.5%	incubate in the dark at 30°C

oxaloacetic transaminase (GOT), phosphoglucomutase (PGM) and malate dehydrogenase (MDH) were investigated and standardized in proper condition of plant ages, plant parts, sample extraction solution, PAGE concentration and staining, in okra, tomato, sweet corn and *Brassica* (Table 2).

Esterase extracted from leaves of 5- day old okra seedling showed more detail of banding pattern than that extracted from root (Figure 1). Esterase extracted from leaves of 7- day old okra seedling showed more detail of banding pattern than that extracted from leave of 7- day old seedling could show differences among varieties (Figure 3) during esterase extracted from seed showed no difference among varieties (Figure 4). It had been reported by Stegman (1973) and Hosaka (1985) that age and specific organ of plants influenced their protein and isozymes banding patterns. The results of this experiment agree with the above reports. Banding pattern of acid phosphatase extractions (Figure 5) was made aviable to show the difference among varieties but not as well as esterase.

GOT, MDH and esterase and peroxidase could show differences in some varieties of tomato (Figure 6 and Figure 7). Hasen, 1992 succeed to identify egg plant cultivars by using seed protein, but our experiment could not find any major difference among varieties of tomato seed protein bands (Figure 8). Although one band showed variation among some varieties, but not enough to characterize all cultivars.

It had been found that extraction buffer also effected isozymes banding pattern. Peroxidase extracted with phosphate buffer show difference electrophoresis banding pattern from that extracted from Tris buffer (Figure 9). Peroxidase extracted with phosphate buffer show difference between varieties of sweet corn Argo sweet (Argo T and ST) and Super sweet (Thai composit super sweet DMR) while those extracted with Tris buffer show no difference.

Esterase could identify the difference of F1-hybrid from male plant in chinese cabbage (Figure 10). Anyhow, more than one isozymes are suggested

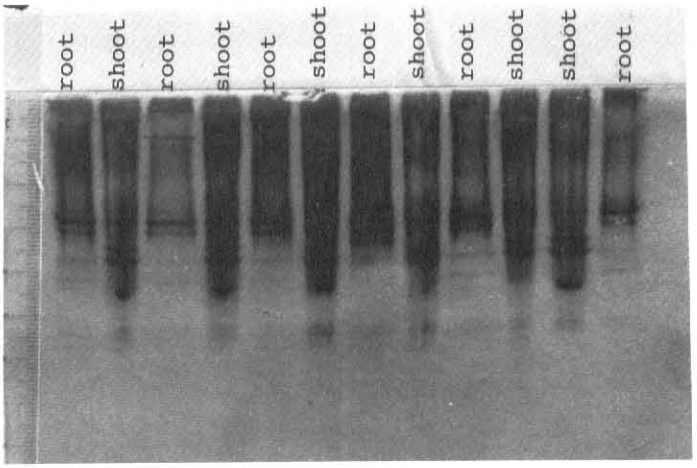

Figure 1 Banding patterns of esterase extracted from root and shoot of 5-day old okra seedlings.

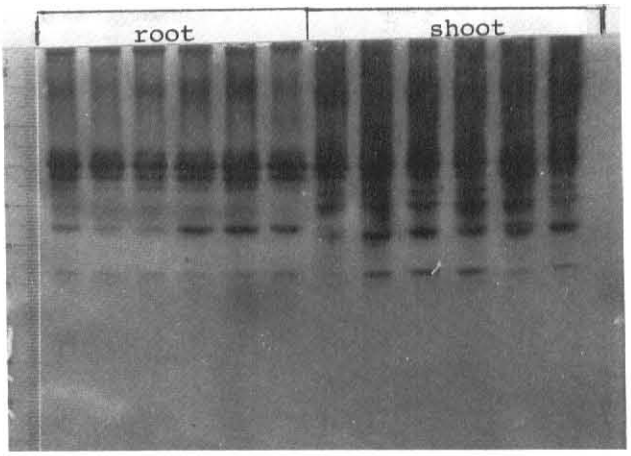

Figure 2 Banding patterns of esterase extracted from root and shoot of 7-day old okra seedlings.

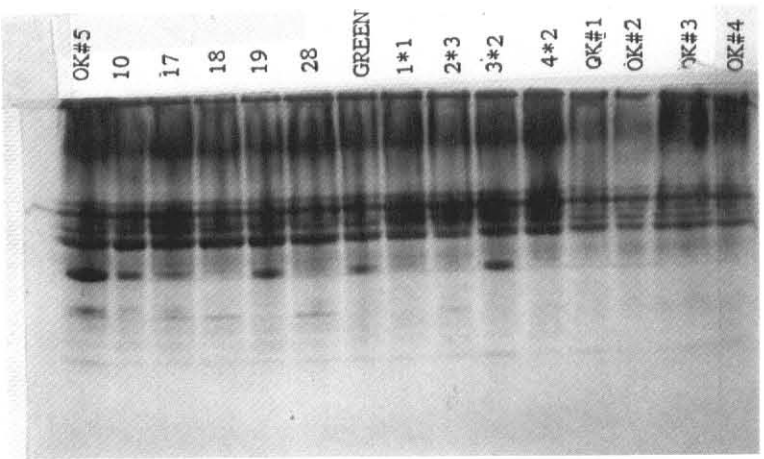

Figure 3 Banding patterns of esterase extracted from leaves of 7-day old okra seedlings.

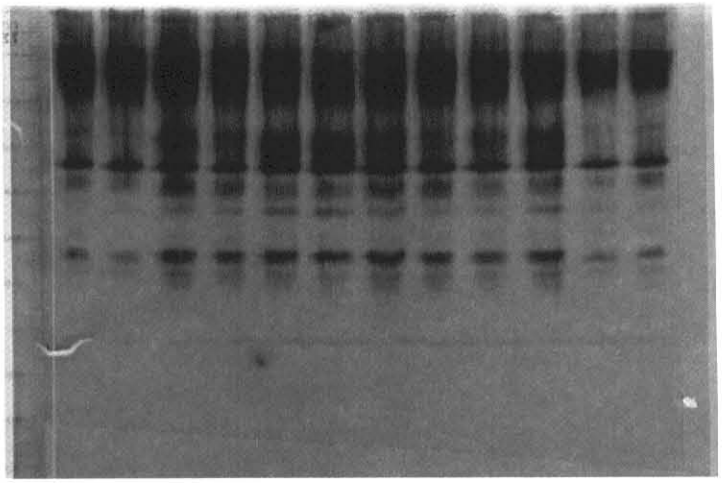

Figure 4 Banding patterns of esterase extracted from okra seed at 2-day imbibition period.

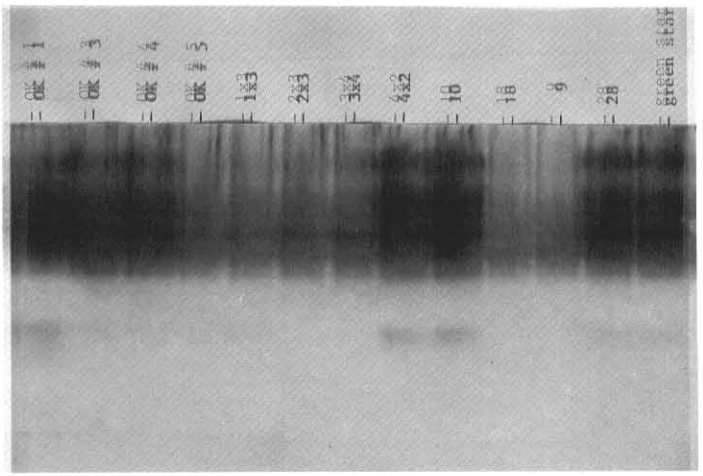

Figure 5 Banding patterns of acid phosphatase extracted from leaves of 7-day old okra seedlings.

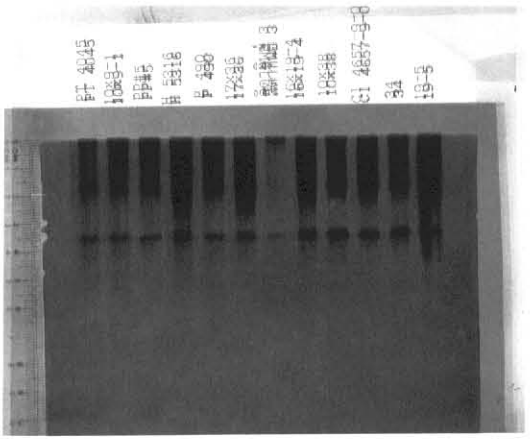

Figure 6 Banding patterns of esterase extracted from leaves of 7-day old tomato seedlings.

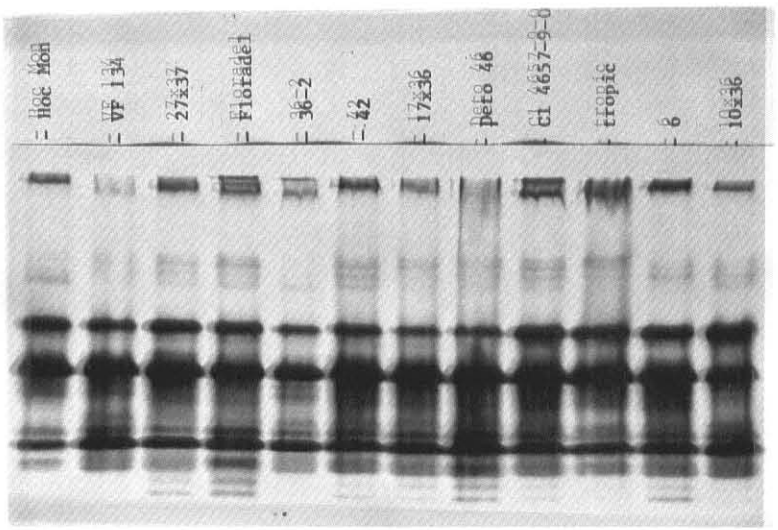

Figure 7 Banding patterns of peroxidase extracted from leaves of 7-day old tomato seedlings.

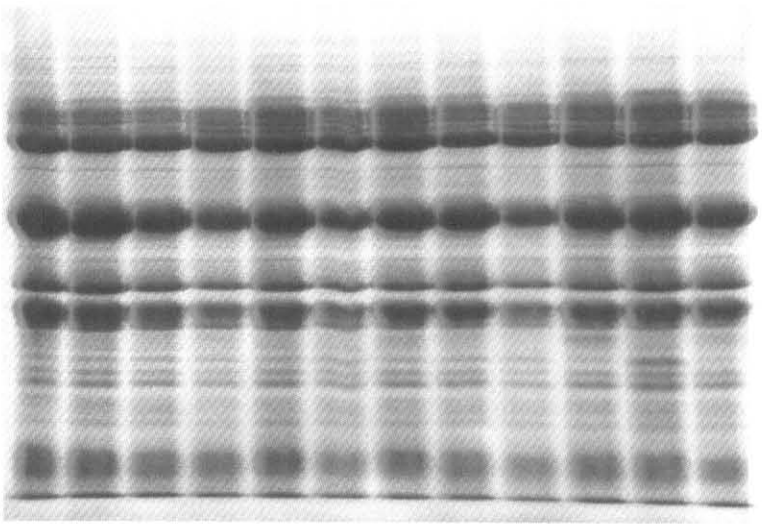

Figure 8 Banding patterns of protein extracted from leaves of 7-day old tomato seedlings.

to be analysed to sufficiently distinguish the different electrophoresis banding pattern in *Brassica*.

It is concluded that peroxidase in sweet corn, ADH and esterase in *Brassica;* esterase in tomato and okra showed the best expression of banding patterns in showing the differences among some breeding lines. Anyway, protein and isozymes banding patterns are unique for each species. Although isozymes have been successfully to distinct different among species (Sundberg and Glimelius, 1986; Hossain, 1990; and Hasen 1992), there is insufficient polymorphic to distinct the differences within species for all varieties as shown in our experiments.

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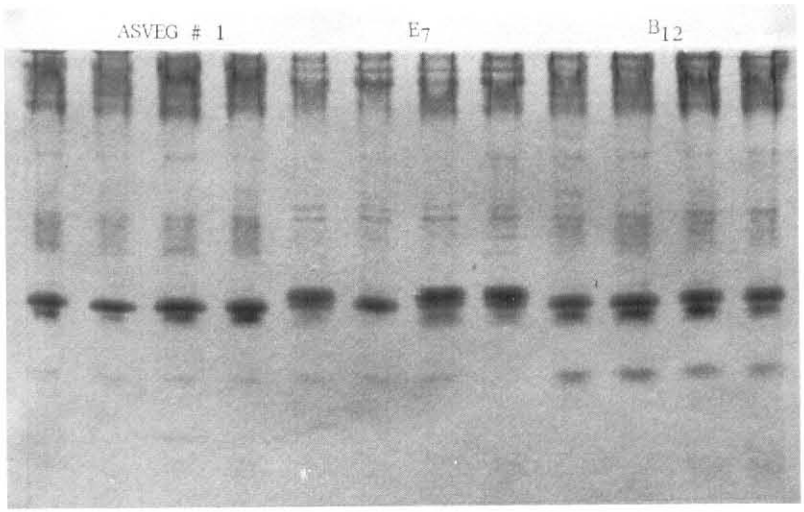

Figure 9 Banding patterns of esterase extracted from leaves of 7-day old chinese cabbage seedling; male, female and F1-hybrid.

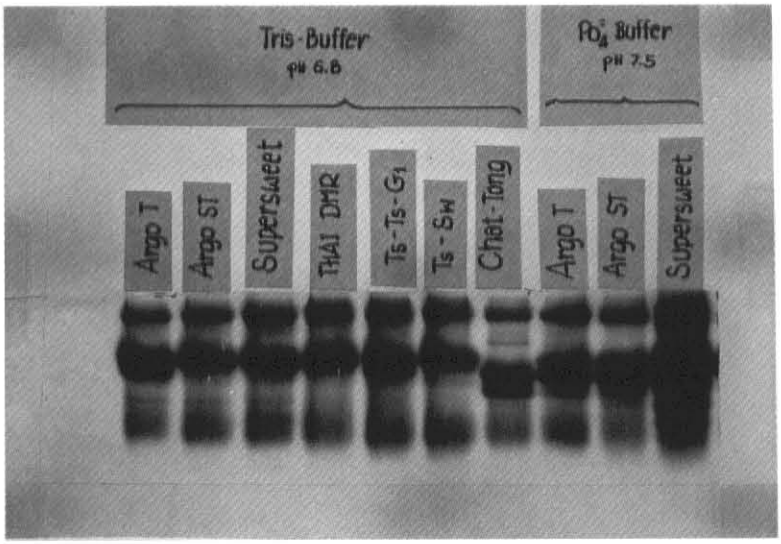

Figure 10 Banding patterns of peroxidase extracted from leaves of 7-day old sweet corn seedlings, comparing between Tris- and Phosphate extracted buffer.

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