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Electrophoretic Variation and Enzyme Storage Stability in Cucumber

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Abstract. **Forty-seven enzymes and general protein of fruit, seed, and cotyledons of *Cucumis sativus* L. var. *sativus* and var. *hardwickii* (Royle) Kitamura were examined by horizontal starch gel electrophoresis to provide information on enzyme activity, stability, resolution, and variability. Of the 5 extraction buffers tested, a Tris base/Tris HC1 buffer at pH 7.1 provided consistent, clear resolution of the enzymes tested. Further studies of several enzymes revealed: 1) 30% DMSO buffer extended the storage life of phosphogluconate dehydrogenase (PGD); 2) the appearance of additional bands to glucosephosphateisomerase (GPI) and disappearance of bands from PGD zymograms occurred when extracts were stored for 7–8 days at 10°C; 3) the zymogram patterns of glutathione reductase (GR), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), and phosphoglucomutase (PGM) were not affected under this storage regime; and 4) zymograms of cotyledonary tissue of seedlings grown under flourescent light in either a 9 or 12 hr light photoperiod at 30°/26° and 22°/18° day/night temperatures for 14 days then stored for 120 days at −18° were similar to fresh extracts. Polymorphisms were observed in GPI, GR, IDH, peptidase, PGD, and PGM. No variety specific electromorphs were observed between *hardwickii* and *sativus*, thus supporting the conspecific hypothesis regarding these 2 varieties. Twenty-four of 46 plant introductions and 5 of 11 inbred lines were variable for 1 or more of the 6 polymorphic isozymes.**

Recently, several enzymes have been investigated in the horticulturally important genus *Cucumis* (2, 9, 15). These and others have proven useful in studying the chemistry, biosystematics, and evolution of this genus (10, 20, 21, 22, 23, 24, 26, 29, 31, 35, 38). Isozymes have been used as biochemical genetic markers in a wide variety of organisms. The mendalian nature of allozymic variation provides the analytic and predictive power of this methodology. Of particular interest to plant breeders and geneticists are its capacities to reflect levels of variability within cultivars or varieties, identify cultivars or varieties, confirm and quantify hybridization, monitor mating system parameters, and identify marker genes linked to single genes of commercial importance, such as disease resistance (37).

Limited information exists on linkage relationships and gene mapping in *Cucumis*. Nearly 100 simply inherited genetic traits have been catalogued in cucumber (30), but many are difficult to assess without growing mature plants and/or progeny testing. It would be useful, therefore, to obtain genetic markers which permit the characterization of linkage groups and, eventually, the development of a chromosome map for species within this genus. Nevertheless, in some instances, the conditions of plant growth and storage of plant extracts play significant roles in isozyme electrophoretic mobility (12, 16). Therefore, before the inheritance of any enzyme is investigated, the extent to which environmental and handling factors affect isozyme mobility should be determined.

Since information on electrophoretic variation in cucumber is limited, this study was initiated to: determine suitable electrophoretic extraction and operational buffers; obtain information concerning the activity of a diverse group of enzymes in various plant tissues; characterize isozyme variation in *C. sativus*; and examine the stability of isozymes under different growing conditions and storage regimes.

Materials and Methods

Buffer specificity and enzyme activity. Mature fruit, seed, and cotyledons of 2 inbred *C. sativus* L. var. *sativus* lines (GY2 and USDA 1379) and one *hardwickii* (Royle) Kitamura collection (PI 183967) were used to assess tissue enzyme activity. Hereafter, these 2 botanical varieties will be referred to as *sativus* and *hardwickii*, respectively. Whole seeds were sampled as both imbibed (aerated in water at 32°C for 12 hr) and sprouted (aerated in water at 32° for 36 hr or until radicle emergence of 5 mm occurred). Cotyledons of 5–7 individuals within a line or collection were harvested from 14-day-old seedlings germinated and grown at about 25° in vermiculite, either under natural light (8 hr average of 71 W/m^2; about 140 μmol s^{-1}m^{-2} using a photoperiod of 8 hr light/16 hr dark) or in darkness.

To determine a suitable extraction buffer, samples of about 3 g of fruit (mesocarp and endocarp, excluding seed, 39 days postpollination), 40 seeds, and 20 cotyledons of each line were ground separately in a mortar and pestle in each of 5 extraction solutions. The solutions (in units per liter) were: 1) 0.67 g Tris base, 7.02 g Tris HCl, pH 7.1; 2) 30% dimethyl sulfoxide (DMSO), pH 8.5; 3) 1.54 g dithiothreitol, 20 g polyvinylprolidone (PVP)-360, 4.44 g Tris HCl, 2.66 g Tris base, pH 8.5; 4) 4.44 g Tris HCl, 2.66 g Tris base, 1 ml 2-mercaptoethanol, 0.06 g EDTA, pH 8.5; and, 5) 5 ml mercaptoethanol, 35.5 g Na_2HPO_4, 30 g NaH_2PO_4, 200 PVP-40. Extraction solution volumes of 0.1, 0.25, and 1.0 to 2.0 ml were added to the 3 g samples of fruit, cotyledons, and seed, respectively, just prior to grinding. Samples were centrifuged 5 min at 1000 × g, and the supernatants were stored at about 5°C for not longer than 30 min before use in horizontal starch gel electrophoresis using the techniques of May et al. (18) and May (19).

Gels consisting of 21 g electrostarch (Electrostarch Co., Madison, Wis.) and 21 g Connaught starch (Connaught Laborato-

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ries, Willowdale, Ontario) were prepared in 300 ml of 4 different buffers. The buffers described by Ridgway et al. (28), Clayton and Tretiak (5), Markert and Faulhaber (17), and Selander et al. (33; buffer 4) were utilized and are referred to as R, C, M, and S, respectively. Filter paper wicks (3 × 8 mm, Schleicher and Schuell No. 470) were dipped into the fresh homogenates and inserted in the gels. A wick dipped in red food coloring (SCM Corporation, Westlake, Ohio; in water, propylene glycol and artificial colors) was used to produce a band which marked the front. An electric potential of 200 V (maximum of 75 mA) was passed through the gels via R, C, and S buffers for 4 to 6 hr. When the dye marker migrated to within 30 mm of the end of the gel, the gel was cut horizontally into 4 slices by drawing a monofilament thread through it. The slices provided the option of staining for 4 different enzyme systems or for reevaluating the same zymograms on several slices. Modified staining solutions (36) of Allendorf et al. (1), Brewer (3), and Shaw and Prasad (34) were applied to visualize isozyme zymogram patterns of 47 enzymes and general protein (Table 1). The relative activity and resolution of each enzyme was noted and enzymes were categorized as being inactive or having activity in which the optical resolution was either adequate or inadequate for possible isozyme analysis, was based on band intensity and clarity.

Isozyme stability. Studies were conducted to determine the degree to which banding patterns changed when seedlings were grown under different environmental conditions and when homogenates were kept under different storage regimes. In the first study, the seeds of 'Addis', 'Aodai-Nazare', 'Calypso', 'Clinton', 'Early Triumph', 'GY-14A', 'Poinsett 76', and 'Sampson' were planted in a completely randomized design with 6 replications in each of 4 environments (2 photoperiods and 2 temperature regimes) in the North Carolina State Univ. Phytotron, Raleigh, N.C. Seedlings were grown for 14 days under either a 9 hr photoperiod (642 μmol $s^{-1}m^{-2}$), or a 12 hr photoperiod consisting of the usual 9 hr with a 3 hr incandescent light (44 μmol $s^{-1}m^{-2}$) night interruption. The temperature of the chambers was either at 30°/26°C or 22°/18°, day/night. Cotyledons then were harvested and stored at about -18° for 30, 60, 90, and 120 days. These samples were compared electrophoretically to samples of freshly-harvested seedlings, and with seedlings that had been stored at -18° for 24 hr.

In the 2nd study, samples of cotyledondary tissue of each cultivar were prepared with extraction solutions 1, 2, and 4 each day for 10 days and held at 10°. On day 10, all samples were examined by electrophoresis and zymograms of GPI, GR, IDH, MDH, PGD, and PGM were compared to patterns recorded in the controlled environment studies.

Electrophoretic variation. An electrophoretic survey was conducted using 19 enzymes which were found to have adequate resolution during enzyme activity studies. Fifty-two hybrids and inbred lines of *sativus*, and 5 *hardwickii* collections were selected on the basis of their morphological and geographical diversity (Table 2). Cotyledons were harvested from 7- to 14-day-old seedlings grown in vermiculite at about 25°C under 8 hr of natural light (8 hr average of 71 W/m^2; about 140 μmol $s^{-1}m^{-2}$) and 16 hr of darkness. Cotyledonary samples were extracted in buffer 1, held at -75° for 4 to 8 days and examined electrophoretically for acid phosphatase (ACP), alkaline phosphatase (AKP), diaphorase (DIA), esterase (EST), fructose diphosphatase (FDP), GPI, glutamic pyruvic transaminase (GPT), GR, IDH, leucine aminopeptidase (LAP), MDH, 4-methylumbelliferyl phosphatase (MUP), peptidase with phenyl-alanyl-proline (PEP-PAP), with leucyl-alanine (Pep-LA), with leucyl-leucyl-leucine (Pep-LLL), phosphogluconate dehydrogenase (PGD), PGM, shikimic dehydrogenase (SKDH), and triose phosphate isomerase (TPI). Isozyme banding patterns of the enzymes were recorded and comparisons were made among zymograms. The nomenclature follows a modified form described by Richmond (27) such that isozymes for the enzymes GPI, GR, IDH, PEP-PAP, PGD and PGM are designated as Gpi, Gr, Idh, Pep-pap, Pgd and Pgm, respectively. Hyphenated numerals refer to multiple isozymes, numbered from most cathodal to most anodal and variants for a particular isozyme are designated by numerals enclosed by parentheses. As an example, a 2-banded individual at the locus Gr-1, is designated Gr-1 (12).

Results

Buffer specificity and enzyme activity. Of the extraction buffers tested, the best isozyme resolution was obtained using buffer 1 (Tris base/Tris HCl). Buffer 2 provided inconsistent, nonsystematic results, occasionally producing blurred, indistinguishable banding patterns, whereas the performance of buffers 3 through 5 was variable depending on the enzyme tested.

There were no apparent intervarietal differences with respect to a tissue's enzyme activity. Electrophoretic examination revealed that 14, 27, 31, 29, and 30 of the 47 enzymes or general protein tested provided isozyme resolution which was potentially acceptable for genetic analysis in fruit, imbibed seed, sprouted seed, and cotyledons grown under light and darkness, respectively (Table 1). Twenty-nine, 16, 12, 14, and 13 enzymes were not active in imbibed seed, sprouted seed, light or dark grown aand cotyledons, respectively. Of the plant parts examined, extracts taken from fruit provided the poorest resolution and produced the highest frequency of enzyme inactivity. Only 29% of the enzymes surveyed in fruit produced banding patterns with adequate resolution for possible interpretation, whereas approximately 60% were undetectable. The remaining 11% provided activity, but isozymes were not adequately resolveable for analysis. In contrast, when results from seed and cotyledons were considered collectively, enzyme inactivity and enzymes producing zymograms with acceptable resolution ranged between 33% to 56% and 25% to 65%, respectively. Cotyledons were the tissue of choice for subsequent studies because of their homogeneous nature, high level of enzyme activity, clearly resolveable isozymes, ease of handling, and rapid availability. When considering all 32 active enzymes, the S buffer system provided the clearest zymogram resolution in 37% of the cases, followed by R (33%), C (24%), and M (6%).

Isozyme stability. No intravarietal differences in isozyme zymogram patterns were observed for GPI, GR, IDH, MDH, PGD, and PGM from the 8 cultivars tested over the 4 environments. After 120 days of storage at -18°C, enzyme activity from cotyledon extracts was similar to those held for 30 days. Zymogram phenotypes were identical to those produced from fresh extracts or from cotyledons held at -18° for 24 hr before extraction. When cultivar samples which had been prepared in 3 extraction buffers were evaluated after 10 days storage at 10°, differences in zymograms were apparent. Zymograms of PGI from samples prepared in each of 3 extraction solutions (1, 2, and 4) showed progressive addition of bands starting on day 8 of storage. For example, the zymogram of 'Addis', which was a single band in freshly extracted samples, was resolved into 2-bands on day 8, and into 3-bands on day 10. While extraction solution 2 provided the best resolution, blurring of zymograms began after samples were stored 24 hr and extracted in solution

Table 1. Relative activity and electrophoretic resolution of 47 general metabolic enzymes and general protein observed in *Cucumis sativus* fruit, seed and cotyledons.

Enzyme	Abbreviation	*C. sativus* tissue						
		Seed			Cotyledons[z]		Gel buffer[y]	
		Fruit	Sprouted	Imbibed	Light grown	Dark grown	Best	Alternate
Aspartate aminotransferase	AAT	1[x]	1	1	1	1	M	R
Acid phosphatase	ACP	2[w]	2	2	1	1	S	–
Adenosine deaminase	ADA	2	2	2	2	2	–	–
Alcohol dehydrogenase	ADH	2	1	1	2	1	C	M
Aldolase	ALD	2	1	1	1	1	S	C
Adenylate kinase	AK	1	1	1	1	1	S	C
Alkaline phosphatase	AKP	2	1	2	1	1	S	–
Catalase	CAT	2	1	2	2	2	S	–
Creatinine phophokinase	CPK	2	1	1	1	1	C	–
Diaphorase	DIA	2	1	1	1	1	R	S
Esterase	EST	1	1	1	1	1	R	C
Fructose diphosphatase	FDP	2	1	1	1	1	R	C
Fumarase	FUM	2	1	1	2	2	C	S
Galactosaminidase	GAM	3[v]	3	3	3	3	–	–
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH-NADP	3	3	3	3	3	–	–
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH-NAD	2	1	1	1	1	M	C
Glutamine dehydrogenase	GDH-NADP	2	1	1	1	1	C	–
Guanine deaminase	GDA	2	2	2	2	2	–	–
B-glucosidase	B-GLU	2	1	1	1	1	R	C
Glucose-6-phosphate dehydrogenase	G6PDH	2	1	1	1	1	C	–
Glucosephosphateisomerase	GPI	1	1	1	1	1	R	C
Glutamic pyruvic transaminase	GPT	2	1	1	1	1	S	–
Glutathione reductase	GR	2	1	1	1	1	R	S
Glucoronidase	GUS	1	2	2	1	1	R	M
Hydroxybutyric dehydrogenase	HBDH	2	2	2	2	2	–	–
Isocitrate dehydrogenase	IDH	1	1	1	1	1	S	–
Lactate dehydrogenase	LDH	2	2	2	2	2	–	–
Leucine aminopeptidase	LAP	1	1	1	1	1	S	C
Manatol dehydrogenase	MADH	2	2	2	2	2	–	–
Malate dehydrogenase	MDH	1	1	1	1	1	C	–
Malic enzyme	ME	2	2	2	2	2	–	–
4-Methylumbelliferyl phosphatase	MUP	2	1	2	1	1	S	–
Octanol dehydrogenase	ODH	2	1	1	2	2	C	S
Peptidase with glycyl-leucine	PEP-GL	3	3	3	3	3	–	–
Peptidase with leucyl-alanine	PEP-LA	1	1	1	1	1	R	–
Peptidase with leucyl-leucyl-leucine	PEP-LLL	1	1	1	1	1	R	–
Peptidase with phenyl-alanyl-proline	PEP-PAP	1	1	1	1	1	S	C
Peroxidase	PER	2	2	2	2	2	–	–
Phosphogluconate dehydrogenase	PGD	1	1	1	1	1	S	–
Phosphogylcerate kinase	PGK	3	3	3	3	3	–	–
Phosphoglucomutase	PGM	2	1	1	1	1	S	C
Pyruvic kinase	PK	3	3	3	3	3	–	–
General protein	PRO	2	1	2	1	1	R	–
Sorbitol dehydrogenase	SDH	2	2	2	2	2	–	–
Shikimic dehydrogenase	SKDH	1	1	1	1	1	C	S
Succinate dehydrogenase	SCDH	2	2	2	2	2	–	–
Triose phosphate isomerase	TPI	1	1	1	1	1	–	–
Xanthine dehydrogenase	XDH	2	2	2	2	2	R	C

[z]Cotyledons green ad etoliated germinated and grown under natural light (about 70 W/m^2/hr; 8 hr light/16 hr dark) and cotyledons grown in dark, respectively, for 14 days.

[y]R = Ridgway, C = Clayton and Tretiak, M = Market and Faulhaber, S = Selander and refers to citations made in text.

[x]1 = activity with adequate resolution for possible isozyme analysis.

[w]2 = no activity.

[v]3 = activity with inadequate resolution for possible isozyme analysis.

Table 2. Electrophoretic variation observed for glucose phosphatase (GPI), glutathione reductase (GR), isocitrate dehydrogenase (IDH), peptidase with phenyl-alanyl-proline (PEP-PAP), phosphogluconate dehydrogenase (PGD), and phosphoglucomutase (PGM) in 52 *C. sativus* var. *sativus* and 5 var. *hardwickii* collections.

Name	Source or origin	Assigned nomenclature for electrophoretic phenotypes of enzymes[z]						
		Gpi	Gr-1	Gr-2	Idh	Pep-pap	Pgd	Pgm
		'Sativus'						
Addis	NCSU	11	11	11	11	11	12	22
Aodai-Nazare*	Asgrow	11	11	11	11	11	11	11
Ashley	Asgrow	11	11	11	11	11	22	11
Clinton*	NCSU	11	11	11	11	11	11	11
Colet	Royal Sluis	11	11	11	11	11	11	11
Corona	Van Den Berg	11	11	11	11	11	22	22
G-76*	NCSU	11	11	11	11	11	12	12
GY-2*	NCSU	11	11	11	11	11	11	22
GY-14*	NCSU	11	11	11	11	11	11	22
GY-14*	UW	11	11	11	11	11	12	22
GY-57U*	NCSU	11	11	11	11	11	12	11
Marbel	Royal Sluis	11	11	11	11	11	11	11
Poinsett* 6	Arco Seed	11	11	11	11	11	12	11
Riesen-schall	Royal Sluis	11	11	12	11	11	22	22
Sampson	NCSU	11	11	11	11	11	11	12
Spring 440	Asgrow	11	11	11	11	11	22	11
Tamor	Asgrow	11	11	11	11	11	12	12
Tempo	Harris	11	11	11	11	11	11	22
Early Triumph	Peto	11	11	11	11	11	22	11
USDA 1379*	USDA/ARS	11	11	11	11	11	12	22
USDA 1397*	USDA/ARS	11	11	11	11	11	22	22
USDA 1909*	USDA/ARS	11	11	11	11	11	22	11
USDA 1983*	USDA/ARS	11	11	11	11	11	22	11
PI 114339	Japan	11	11	11	11	11	11	11
PI 118279	Brazil	11	11	11	11	11	11	12
PI 135123	New Zealand	11	11	11	11	11	22	11
PI 135345	Afghan-istan	11	11	11	11	11	12	12
PI 137853	Iran	11	11	11	11	11	12	12
PI 164743	India	12	11	22	11	11	12	22
PI 167197	Turkey	11	11	11	11	11	12	12
PI 177363	Syria	11	11	11	11	11	12	12
PI 177364	Iraq	11	11	11	11	11	22	11
PI 183224	Egypt	11	11	11	11	11	22	22
PI 181755	Lebanon	11	11	11	11	11	12	12
PI 188807	Philip-pines	11	12	12	11	11	22	11
PI 192940	China	11	11	11	11	11	11	11
PI 200815	Burma	33	12	11	11	12	11	11
PI 205996	Sweden	11	11	11	11	11	12	12
PI 211589	Israel	11	11	11	11	11	12	12
PI 212059	Greece	11	11	11	11	11	22	12
PI 212233	Japan	11	11	11	11	11	12	12
PI 217946	Pakistan	11	11	11	11	11	12	11
PI 220860	Korea	11	11	11	11	11	22	12
PI 249561	Thailand	11	11	12	11	11	12	11
PI 255933	Nether-lands	11	11	11	11	11	22	11
PI 257486	China	11	11	12	11	11	22	12
PI 263046	USSR	11	11	11	11	11	12	11
PI 283901	Czecho-slovakia	11	11	11	11	11	12	12
PI 288238	Egypt	11	11	11	11	11	22	11
PI 288994	Hungary	11	11	11	11	11	12	12
PI 355055	Iran	11	11	11	11	11	22	12
PI 390266	Japan	11	11	11	11	11	11	11
		'Hardwickii'						
PI 486336	India	11	11	11	11	11	22	11
PI 462369	India	11	11	11	11	11	11	11
PI 183967	India	11	11	11	33	22	11	11
PI 215589	India	11	22	11	33	22	11	11
LJ 91176	India	11	11	11	11	22	11	11

[z]Inbreds are indentified by "*".
[y]NCSU = North Carolina State Univ., UW = University of Wisconsin, USDA/ARS, Madison, Wis.
[x]Electromorphs for isozyme 11 in all cases were given the mobility designation 100. All other isozymes produced protein products with relative mobilities to electromorph 100 as follows; Gpi(2)-118, Gpi(3)-88, Gr-1(2)-89, Gr-2(2)-89, Idh(3)-78, Pep-pap-2(2)-106, Pgd(2)-106, Pgm(2)-88.

4. Blurring persisted for 72 hr of storage, then disappeared, leaving distinct isozymes similar to those in fresh extracts.

Disappearance of PGD bands occurred as a result of storage after extraction with solutions 1 and 4. After 24 hr storage, for instance, zymograms of samples from 'Addis' extracted in solution 4 showed loss of one band when compared to freshly extracted samples which were 2-banded. On day 7, zymograms of samples extracted in solution 1 were observed to resolve a single form, whereas those extracted in solution 2 were resolved as 2 forms on day 9. Nevertheless, the resolution of PGD zymograms from samples extracted in solution 2 on day 10 was not adequate to provide consistent classification of individuals as 2-banded phenotypes. The resolution of samples prepared in extraction buffers 1 and 2, held for 10 days, then stained for GR, IDH, MDH, and PGM was comparable to those of freshly extracted samples. Patterns resulting from samples extracted with solution 4 were blurred and unresolvable after 48 hr storage.

Electrophoretic variation. An electrophoretic survey of 57 accessions of *Cucumis sativus* revealed that they were monomorphic for Acp-2, Akp, Dia-2, Est-4, Fdp-2, Gpt-1, Lap, Mdh, Mup-1, Pep-pap-1, Pep-la-1, Pep-lll-1, 2, Pgd-1, Skdh, and Tpi in which zymograms were single banded. Isozyme variation was determined for Gpi, Gr-1, Gr-2, Idh, Pep-pap-2, Pgd-2, and Pgm (Table 2). All individuals were monomorphic for the Gpi ll except for PI 164743 and PI 200815, which were determined as Gpi 12 and 33. Gr-1 variants (phenotypes) other than 11 were

12 (PI 188807 and 200815) and 22 (PI 215589). For Gr-2 the variants 12 (Riesenschall, PI 188807, 249561 and 257486) and 22 (PI 164743) were observed, in addition to Gr-2 (11). All individuals were monomorphic Idh (11) except for the 2 *hardwickii* collections, PI 183967 and 215589, which were 33. Similarly, only 3 *hardwickii* collections (PI 183967, 215589, and LJ 91176) were monomorphic Pep-pap-2 (22) and 1 *sativus* (PI 200815) was 2-banded (12), whereas all other individuals were Pep-pap (11). The broadest range of variant distribution was observed in PGD and PGM. The frequency of the Pgd-2 variants (11, 12, 22) among the individuals surveyed was 17, 21, and 19, respectively, whereas frequencies for the same variants at Pgm were 28, 18, and 11, respectively.

Discussion

Although the performance of the Tris base/Tris HC1 extraction buffer proved superior to the other buffers tested, observations suggest that buffers containing DMSO may be useful in extending the storage life of selected, relatively unstable cucumber enzymes, such as PGD. DMSO is an organic solvent which, by altering the hydrophilic nature of the enzyme, allows it to maintain activity for prolonged periods (12 to 20 hr) at moderate temperatures (37°C) in high (35% to 60%) solvent concentrations (25).

Higher resolution and activity in fruit tissue may be obtained by concentrating enzymes. Moreover, modifying the extraction medium or operational buffers may improve the resolution of active enzymes which produced zymograms unacceptable for interpretation. From a methodological standpoint, the use of cotyledonary or seed tissue provides the advantage of screening large numbers at an early age in a small area, while retaining high resolution and activity.

The disappearance or appearance of additional bands during storage is not unique (11, 13, 14, 36) and is most easily explained by in vitro alterations of the enzymes. These changes may result from a number of posttranslational alterations which can reduce the activity or modify the structure of the enzyme. These posttranslational changes include deamination, partial proteolysis, nonproteolytic alterations, addition of foreign groups, and disulfide/hydrogen bridging (7, 35). The formation of "secondary" GPI isozymes, resulting in addition of bands, has been observed during the storage of plant extracts (13, 36) and has been shown to be pH and temperature dependent (7). Proteolysis of hexokinase isozymes has produced similar changes in mammalian organisms (4). Blurring of bands may be associated with alterations in the tertiary structure of the isozymes resulting in loss of electrophoretic resolution. The fact that the resolution of zymograms of samples extracted in 2-mercaptoethanol was restored after 72 hr storage at 10°C indicates these changes were transitory and are thus not likely due to disaggregation or aggregation of the original enzyme molecule.

Alterations in banding patterns, such as those observed in this study, are important from the viewpoint of genetic interpretation and analysis. An enzyme's storage dynamics should be known such that posttranslational modifications of proteins, occurring as a result of handling procedures before and after sample extraction, can be prevented. The isozymes of the particular enzymes examined in this study were not affected by various temperature or photoperiod regimes. Moreover, these enzymes appear to be under rather strict genetic control and therefore should be useful as genetic markers.

The amount of electrophoretic variation observed among the individuals surveyed suggests that the genetic diversity in *C. sativus* L., for those enzymes examined, is limited. These findings also support the hypothesis of Dane (6) and Esquinas-Alcazar (8) regarding the conspecific nature of *sativus* and *hardwickii*. Nevertheless, a difference exists at the Idh locus between some *hardwickii* collections and *sativus* individuals surveyed. *Hardwickii* collections PI 183967 and 215589 contain an isozyme form [IDH (33)] which is not present in the *sativus* individuals examined. Likewise, *sativus* contains an isozyme, Gr-2 (22), which does not exist in any *hardwickii* collection examined, and the frequency of Pep-pap-2 (22) is far greater in *hardwickii* than in *sativus*. These and other results indicate that *hardwickii* may be a primitive form of *sativus*, and has retained some morphological (32) and isozyme dissimilarities which may prove useful as genetic markers.

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