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A Study of Selected Isozymes in *Capsicum baccatum*, *Capsicum eximium*, *Capsicum cardenasii* and Two Interspecific F₁ Hybrids in *Capsicum* Species

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Abstract : Selected isozymes were investigated in plants of *Capsicum baccatum* L. (*Solanaceae*) accessions SA219 (P. G. Smith), Hawkes 6489 (P.G.Smith), *Capsicum cardenasii* Heiser and Smith accession SA268 (P.G.Smith), *Capsicum eximium* A.T.Hunz accession Hawkes 3860 (J.G.Hawkes) and two interspecific F₁ hybrids, *C. baccatum* SA219 x *C. eximium* Hawkes 3860 and *C. baccatum* Hawkes 6489 x *C. cardenasii* SA 268. The standard technique of horizontal gel electrophoresis was employed. The gel was cut into several slices and stained for different enzyme systems (aconitase, alanin aminopeptidase, esterase, glutamic-oxaloacetic transaminase, glycerate-2-dehydrogenases, isocitrate dehydrogenase, malate dehydrogenase, peroxidase, phosphoglucomutase, phosphoglucose isomerases and shikimate dehydrogenases). After fixing the gel slices in 50% aqueous glycerol, the number and position of stained bands were recorded.

Key Words: *Capsicum baccatum*, *Capsicum eximium*, *Capsicum cardenasii*, isozymes, electrophoresis.

***Capsicum baccatum*, *Capsicum eximium*, *Capsicum cardenasii* ve Türler Arası F₁ Hibridlerinden İki Üzerinde Yapılan İzoenzim Çalışmaları**

Özet : Seçilmiş izoenzimler *Capsicum baccatum* L. (*Solanaceae*) SA219 (P.G.Smith), Hawkes 6489 (P.G.Smith), *Capsicum cardenasii* Heiser and Smith accession SA268 (P.G.Smith), *Capsicum eximium* A.T.Hunz Hawkes 3860 (J.G.Hawkes) ve türler arası F₁ hibridlerinden ikisi üzerinde çalışılmıştır. Çalışmada standart jel elektroforez metodu kullanılmıştır. Jel dilimlere ayrılmış ve her bir dilim farklı bir enzim sistemi (Akonitaz, Alanin aminopeptidaz, Esteraz, Glutamik-oksaloasetik transaminaz, Gliserat-2-dehidrogenaz, İzositrat dehidrogenaz, Maleyt dehidrogenaz, Peroksidaz, Fosfoglukomutaz, Fosfoglukoz izomeraz ve Şikimik dehidrogenaz) için boyanmıştır. Son olarak da jel dilimleri %50'lik glycerol içerisinde fikse edilmiş ve bant sayıları ile pozisyonları işaretlenmiştir.

Anahtar Sözcükler: *Capsicum baccatum*, *Capsicum eximium*, *Capsicum cardenasii*, izoenzim, elektroforez

Introduction

Morphological markers have several disadvantages when used as markers in botanical studies, e.g., they cannot distinguish homozygotes and heterozygotes from each other, if there is a dominance. Isozymes, on the other hand, generally exhibit Mendelian inheritance, co-dominant expression, complete penetrance and absence of pleiotropic and epistatic interactions. The advantages of these properties were emphasised in early papers on enzyme heterozygosity in *Drosophila* (1,2).

In studies on isozymes, allelic variation for a particular enzyme can be detected on zymograms as fast and slow bands within a zone on a starch gel. By considering the quaternary structure of the enzyme and the patterns from their F₁ hybrids, one can determine the number of alleles coded by each locus. When there is convincing genetic evidence that the given zone is controlled by the single locus, a symbol is assigned to the gene in accordance with the conventional genetic nomenclature, e.g., mnr-2 in the case of the zone MNR-2.

Previous studies in *Capsicum* L. (*Solanaceae*) (3,4) have given some useful background information about the differences in alleles of enzyme-coding genes in *Capsicum*. For the aconitase enzyme system, two zones of activity, apparently controlled by two different loci, were reported in *Capsicum* (5,6), while one or two zones of activity were reported for other plants (7,8).

Glutamic-oxaloacetic transaminase catalyses the reversible conversion of aspartate and alfa-ketoglutarate to oxaloacetate and glutamate (9). The number of loci coding for these isozymes which could be detected is apparently much influenced by the method of extraction, and the electrophoretic conditions applied. Five loci were reported for *Capsicum* with null alleles in one of these loci (6, 11).

NAD-dependent malate dehydrogenases have dimeric structure and usually display a complex banding pattern due to sub-cellular compartmentalisation, gene duplication, and interaction between intra- and intergeneric subunits (12). Three (or more) zones of activity have been resolved in *Capsicum* (13).

Phosphoglucose isomerases are dimeric enzymes which catalyse the reversible isomerisation of fructose-6-phosphate (14). Diploid plants generally possess two loci for these enzymes. Two zones of electrophoresis activity, apparently encoded by two different loci, were observed in *Capsicum* (5,10).

Phosphoglucose mutases are transferases involved in the conversion of glucose-1-phosphate to glucose-6-phosphate. Plants generally contain two isozymes with monomeric structure, active in cytosol and plastid (14). Two regions of electrophoresis activity have been reported in *Capsicum* (6,11)

In this study, we investigated the genetic control of the isozymes of the enzymes aconitase, alanin aminopeptidase, esterase, glutamic-oxaloactic transaminase, glycerate-2-dehydrogenases, isocitrate dehydrogenase, malate dehydrogenase, peroxidase, phosphoglucomutase, phosphoglucose isomerases and shikimate dehydrogenases, in order to illustrate the use of enzyme polymorphism for the identification of *Capsicum baccatum* L. (*Solanaceae*) accessions SA219, Hawkes 6489, *Capsicum cardenasii* Heiser and Smith accession SA268, and *Capsicum eximium* A.T. Hunz accession Hawkes 3860. The results obtained in this present study were thought to be useful for researchers

dealing with plant systematics and for plant breeders dealing with *Capsicum* breeding.

Materials and Methods

Plant Materials

Capsicum baccatum L. accessions SA219 (P.G.Smith), Hawkes 6489 (P.G.Smith), *C. cardenasii* Heiser and Smith, accession SA268 (P.G.Smith), *C. eximium* A.T. Hunz accession Hawkes 3860 (J.G.Hawkes) and two interspecific F₁ hybrids, *C. baccatum* SA219 x *C. eximium* Hawkes 3860 and *C. baccatum* Hawkes 6489 x *C. cardenasii* SA 268, were used as plant materials.

15-30 mg of leaf material, freshly harvested from new growth, was placed in cooled (0-4°C) perspex wells and macerated in 0.15-0.30 ml of extraction medium which consisted of Trizma base, ascorbic acid, KCl, EDTA, MgCl₂.6H₂O, polyvinylpyrrolidone-40, polyvinylpolypyrrolidone, and β-2-mercaptoethanol (10).

The material was ground to a thick creamy homogenate with a perspex rod. Immediately after extraction, each of wells was covered with a cover slip to prevent oxidation. When all samples had been ground, all cover slips were removed and a paper wick 1.5 mm x 12 mm cut from Whatman chromatography paper was put on top of each extract until saturation.

Electrophoresis

Horizontal starch gel electrophoresis was employed. Gels were made before the extracts were prepared, covered with cling film, and kept overnight at room temperature. One hour before extraction, gels were put into a refrigerator at 4°C, and then loaded with wicks. After preliminary tests of gels containing 10%, 11% and 12% starch, it was decided to use 10% starch.

Enzyme staining and scoring of bands

The gel was taken out of the refrigerator and cut into several slices (normally 5 or 6 slices, each 1.5 mm thick). Each slice was stained for a different enzyme system and left in the appropriate staining solution for the enzyme to be visualised in an oven at approximately 37°C in darkness. The staining solution was then poured off and the gel was rinsed a couple of times with distilled water. Finally, gel slices were fixed in 50% aqueous glycerol and the number and positions of stained bands were recorded. Allele designation followed those of Gonzalez

de Leon and Pickersgill (10), since they studied most of the *Capsicum* species previously.

Results

Aconitase (ACON)

Two zones of activity were observed in this present study. ACON-1 contained a single band assigned to allele *Acon-1*³, whose position was the same in all species or accessions used in this study. In the second region, ACON-2, the three species were represented by two alleles. The two accessions of *C. baccatum* carried a slow band, assigned to allele *Acon-2*³. *C. eximium* had a fast band, assigned to allele *Acon-2*¹. Some of the plants of *C. cardenasii* examined were two-banded heterozygotes for ACON-2 and carried two alleles, *Acon-2*¹ and *Acon-2*². The F₁ hybrids of *C. baccatum* x *C. cardenasii* had two-banded phenotypes, as did the F₁ hybrid *C. baccatum* x *C. eximium*. Therefore, the plant of *C. cardenasii* used as the parent of the *C. baccatum* x *C. cardenasii* hybrid must have been homozygote (see Figure 1).

Alanin Aminopeptidase (AAP)

A single zone of activity was found and, within this zone, differences in band positions were observed (see Figure 1). Both accessions of *C. baccatum* displayed a slow band, assigned to allele *Aap-1*⁴, while *C. eximium* and *C. cardenasii* had a fast band, assigned to the allele *Aap-1*¹. Both F₁ hybrids, *C. baccatum* x *C. eximium* and *C. baccatum* x *C. cardenasii*, had two bands, as expected in plants heterozygous for a gene coding for a monomeric enzyme.

Esterase (EST)

There were multiple regions of activity for this enzyme system (see Figure 1). Region 1 always migrated with the borate front. All accessions and species used in this study gave a single band, the position of which did not vary.

Regions 2, 3 and 4 did not stain very well and bands were poorly resolved. Attempts to improve the resolution and staining were not successful. Thus, these regions were not studied any further.

Region 5 contained bands in two different positions. Both accessions of *C. baccatum* had a fast band, assigned to *Est-5*¹, while *C. cardenasii* and *C. eximium* showed a slow band assigned to *Est-5*². When resolution was good,

both F₁ hybrids, *C. baccatum* x *C. eximium* and *C. baccatum* x *C. cardenasii*, had a three-banded zymotype, showing EST-5 to be dimeric, as reported by previous researchers (6,10).

Region 6 was close to the origin and contained a single band which did not vary in the accessions and species used.

Region 7 was slightly cathodal in position and also contained a single invariant band.

Glutamic-oxaloacetic acid transaminase (GOT)

Two zones of activity were found (see Figure 1). Region 1 contained a single band. While both *C. baccatum* accessions had a fast band, assigned to *Got-1*², *C. cardenasii* and *C. eximium* carried a slow band, assigned to allele *Got-1*³. Both F₁ hybrids, *C. baccatum* x *C. eximium* and *C. baccatum* x *C. cardenasii*, had a three-banded zymotype, representing heterozygosity for a gene coding for a dimeric enzyme.

Region 2 contained a single band, whose position did not vary in any of the species or accessions used in this study.

Glycerate-2-dehydrogenases (G-2DH)

Staining the gel for this enzyme produced a single zone of activity with a single band. The position of this band did not vary in any of the species or accessions used (see Figure 2).

Isocitrate dehydrogenases (IDH)

One zone of activity was found (see Figure 2 and Figure 3). Both *C. baccatum* accessions carried a slow band, assigned to allele *Idh-1*³, while *C. eximium* and *C. cardenasii* had a fast band, assigned to allele *Idh-1*². Both F₁ hybrids had a three-banded phenotype, representing heterozygosity for a gene coding for a dimeric enzyme.

Malate dehydrogenases (MDH)

Two or three zones of activity were found. The first zone of activity was very faint and disappeared in a very short time.

The second zone of activity contained two or three bands for all accessions and species used in this study. However, it was difficult to score whether there were two or three bands in this region, so this region was not studied any further.

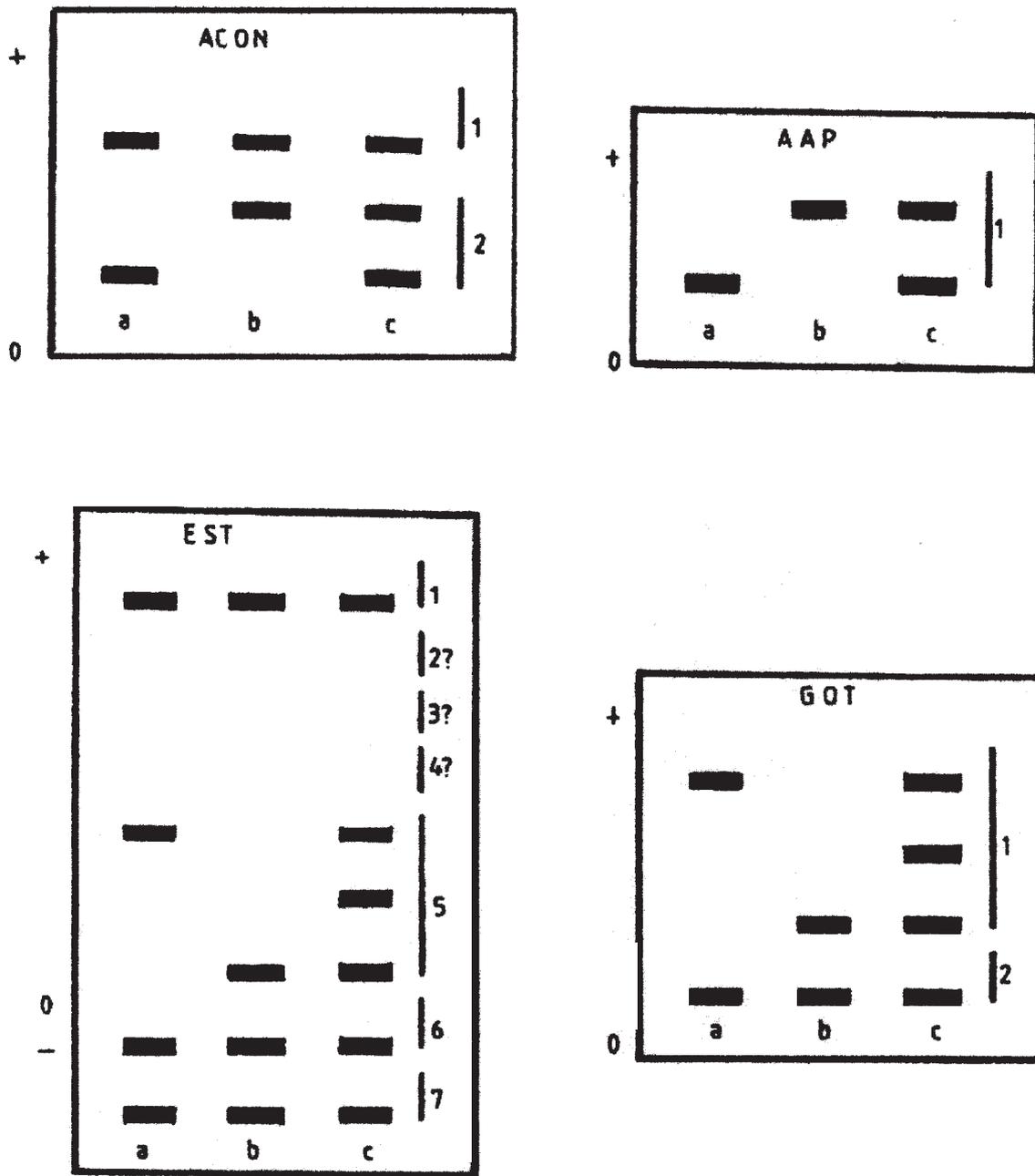


Figure 1. Diagrammatic interpretation of the leaf tissue zymograms of a: *C. baccatum* SA219, b: *C. eximium* Hawkes 3860 and c: F₁ hybrids of *C. baccatum* SA219 x *C. eximium* Hawkes 3860. 0, +, - indicate the origin, anodal and cathodal directions on the gels, respectively.

Since the results were not informative with respect to the controlling loci for MDH, bands for this system were not illustrated.

Peroxidase (PRX)

Two zones of activity were found (see Figure 2). One moved cathodally and the other moved anodally. Both

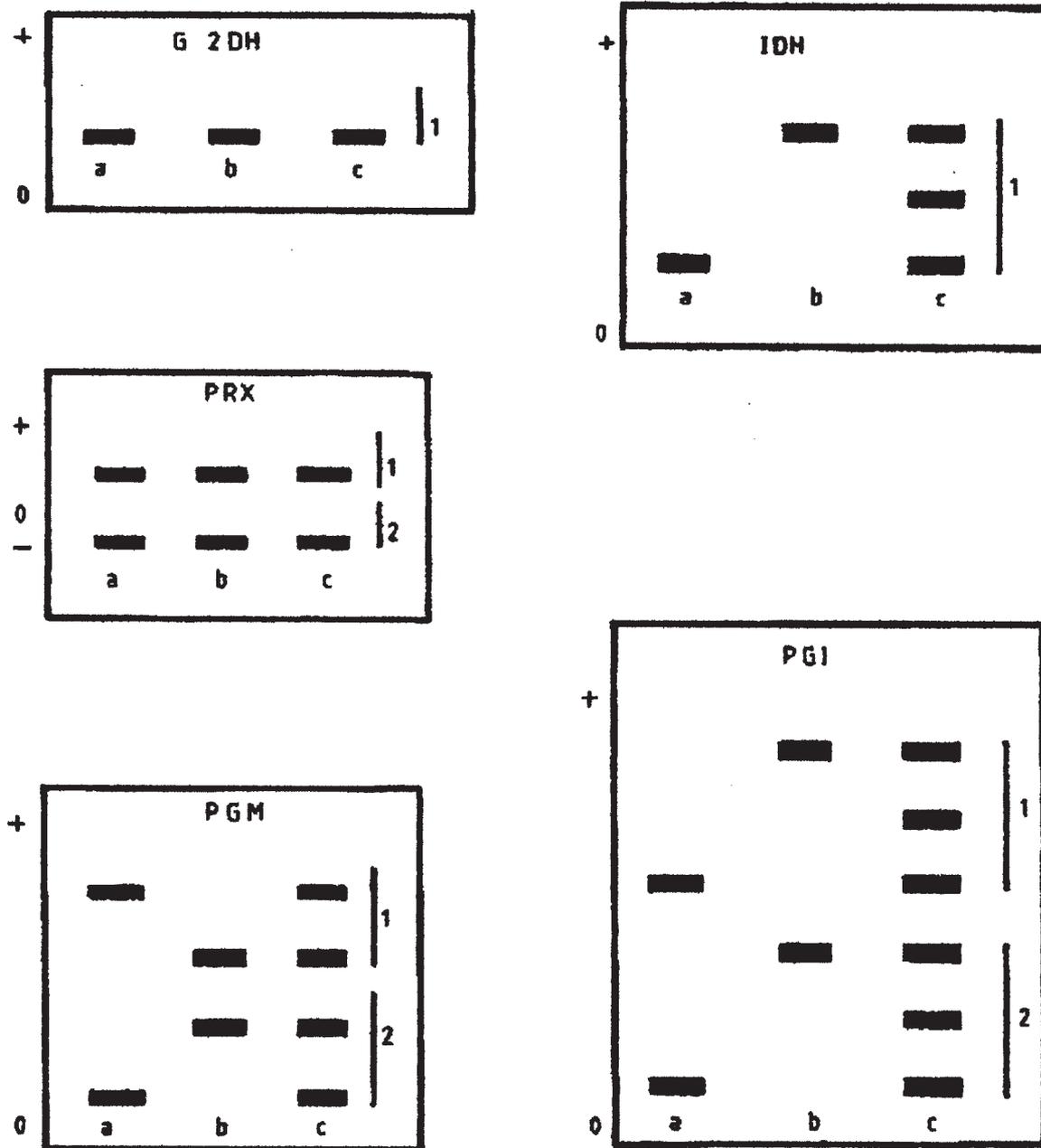


Figure 2. Diagrammatic interpretation of the leaf tissue zymograms of a: *C. baccatum* Hawkes 6489, b: *C. cardenasii* SA268 and c: F_1 hybrids of *C. baccatum* Hawkes 6489 x *C. cardenasii* SA268. 0, +, - indicate the origin, anodal and cathodal directions on the gels, respectively.

zones contained a single band, whose position did not vary in any of the species or accessions used.

Phosphoglucomutase (PGM)

Two zones of activity were found (see Figure 2). PGM-1 contained a single band, whose position varied.

Both accessions of *C. baccatum* had a fast band, assigned to the allele *Pgm-1*¹, while *C. eximium* and *C. cardenasii* carried slightly slower bands, assigned to *Pgm-1*³. Both F_1 hybrids *C. baccatum* x *C. eximium* and *C. baccatum* x *C. cardenasii* had two bands, as expected in plants heterozygous for a gene coding for a monomeric enzyme.

PGM-2 also showed bands in different positions. Both accessions of *C. baccatum* carried a slow band, assigned to the allele *Pgm-2*³, while *C. eximium* and *C. cardenasii* had a fast band, assigned to allele *Pgm-2*². Both F₁ hybrid combinations had two bands, representing heterozygosity for a monomeric enzyme (see Figure 2).

PGM activity can be resolved in two different buffer systems. Gonzalez de Leon and Pickersgill (10) reported that System III gives better resolution, but may produce double bands in one or both regions. The results obtained in the present study were in agreement with those of Gonzalez de Leon and Pickersgill (10) (see Figure 4).

Phosphoglucose isomerases (PGI)

Two zones of activity were found in this study (see Figure 2 and Figure 5). The two accessions of *C. baccatum* carried a slow band in PGI-1, assigned to the

allele *Pgi-1*³, and *C. cardenasii* and *C. eximium* had a fast band, assigned to the allele *Pgi-1*². Both F₁ hybrid combinations had a three-banded phenotype.

The two accessions of *C. baccatum* had a slow band in PGI-2 region, assigned to allele *Pgi-2*⁴, while *C. eximium* and *C. cardenasii* carried a fast band, assigned to allele *Pgi-2*¹. Both F₁ hybrids, *C. baccatum* x *C. eximium* and *C. baccatum* x *C. cardenasii*, had a three-banded zymotype, representing heterozygosity for a gene coding for a dimeric enzyme.

Shikimate dehydrogenases (SKDH)

Two zones of activity were found. The second zone of activity always stained more faintly than the first zone of activity. SKDH-1 contained a single band. Two accessions of *C. baccatum* carried a slow band, assigned to the allele *Skdh-1*⁴, while *C. eximium* and *C. cardenasii* had a fast

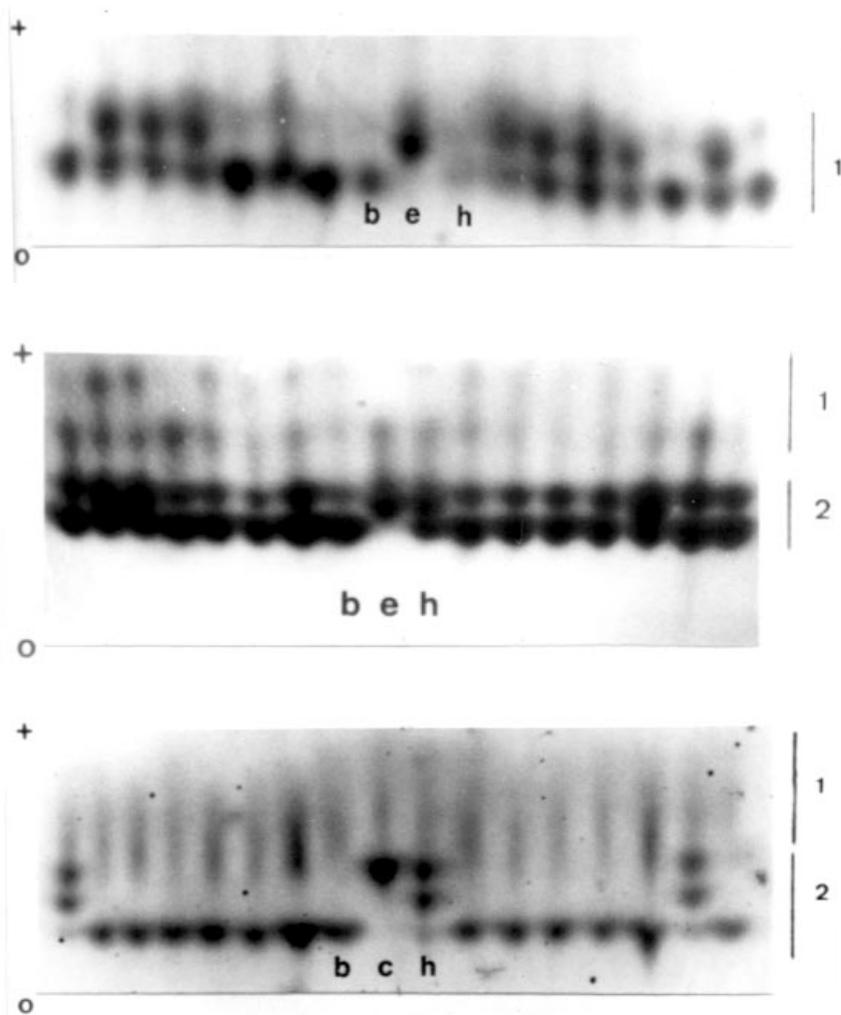


Figure 3. Isocitrate dehydrogenase. Leaf tissue zymograms of b: *C. baccatum* SA219, e: *C. eximium* Hawkes 3860 and h: F₁ hybrids of *C. baccatum* SA219 x *C. eximium* Hawkes 3860. O, + indicate the origin and anodal directions on the gels, respectively.

Figure 4. Phosphoglucose mutase resolved in buffer System III. Leaf tissue zymograms of b: *C. baccatum* SA219, e: *C. eximium* Hawkes 3860 and h: F₁ hybrids of *C. baccatum* SA219 x *C. eximium* Hawkes 3860. O, + indicate the origin and anodal directions on the gels, respectively.

Figure 5. Phosphoglucose isomerase. Leaf tissue zymograms of b: *C. baccatum* Hawkes 6489, c: *C. cardenasii* SA268 and h: F₁ hybrids of *C. baccatum* Hawkes 6489 x *C. cardenasii* SA268. O, + indicate the origin and anodal directions on the gels, respectively.

band, assigned to the allele *Skdh-1*³. Both F₁ hybrids, *C. baccatum* x *C. eximium* and *C. baccatum* x *C. cardenasii*, had a single streak longer than the corresponding band in either of the parents, which would probably consist of two bands.

SKDH-2 had faint bands difficult to score, and so, it was not studied any longer, and, due to the reasons given above, the bands of the system are not shown.

Discussion

Most of the results in the present study are in agreement with previous studies, except those of esterase, isocitrate dehydrogenase and peroxidase.

Although differences between *C. baccatum* and *C. cardenasii* were reported for esterase 7, these differences were not observed in the present study (4).

It has been reported that *C. baccatum* and *C. eximium* have the same alleles for IDH (4, 5). However, the findings of this study are not in agreement with their findings, since these two species had different alleles in the present study.

More than three zones of activity have been reported for peroxidase (6), but only two zones of activity were found in the present study.

Failures to detect the expected number of isozymes for the above-stated enzyme systems can be attributed to pH (15) and/or extraction procedures (16). Changing the buffer systems was found to greatly affect the number of the bands resolved in *Capsicum* (10). In addition to these facts, the variation may be due to changes in the physiology of the plant during the extraction time. The results showed that age of the plant and changes in the environment may affect the physiology of the plant. For example, temperature has been shown to affect isozyme banding patterns in the mullet fish (17).

To overcome these problems the plants should ideally remain at a constant temperature, and the amount of water given should be standardised. Chemical spraying should be monitored and leaves should be checked for damage by viruses.

In this present study, no variation was shown by cultivated accessions from *C. baccatum*, though they

were collected from different localities, which suggested that cultivated species possess less isozymic variability than wild species, as shown by *C. chacoense* in early studies (3,4). The present results obtained for two *C. baccatum* accessions also confirm the facts pointed out by Pickersgill et al. (18), who report that most cultivated peppers in South America are grown in small gardens and each year's crop comes from seeds harvested from the past year's crop. This cycle (planting, harvesting and then planting a small part of the harvest) causes severe genetic bottlenecks and results in reduced variation in these taxa.

C. eximium and *C. cardenasii* are two different species and both in the *C. pubescens* complex. They are easily separable morphologically. However, the results in the present study show that they are not separable isozymically. Similar findings have been obtained in the *C. annuum* complex. This complex can be recognised fairly reliably on morphological grounds, yet isozymic data depict these taxa as being separable (4,5). In these cases, as Gottlieb (19) stated, more weight should be given to evidence of electrophoretic difference than to evidence of electrophoretic similarity, since electrophoretic data tend to underestimate the amount of genetic difference.

Jensen et al. (20) did not find any isozymic variation between *C. cardenasii* and *C. eximium*, as was found in this present study. In their study, there was no basis for recognising *C. cardenasii* and *C. eximium* as separate species, and each analysis involving these taxa indicated that there was a complete overlap between these two. They, therefore, suggested that among the purple flowered taxa three groups be recognised: *C. cardenasii-C. eximium*, *C. pubescens* R. & P. and *C. tovarii* Eshbaugh, Smith & Nickerent.

Electrophoretic studies indicated that *C. eximium* and *C. cardenasii* had more alleles in common with each other than they had with *C. baccatum*. These results indicate that *C. baccatum* is electrophoretically distinct from *C. eximium* and *C. cardenasii*. These results also support the division which is based predominantly on corolla colour and morphological characters and shows that while *C. eximium* and *C. cardenasii* belong to the purple-flowered species group, *C. baccatum* belongs to the white-flowered species group.

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