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ISOENZYME VARIATION IN *CUCUMIS* SPP.

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ABSTRACT

Isoenzyme variation in 13 species of *Cucumis*, including *C. melo* and *C. sativus*, was studied for three enzyme systems, viz. peroxidase, GOT and GDH. The species showed very little similarity for PRX and GDH zymograms. However, there was high degree of similarity among the species for GOT zymogram. All the species possess a genus-specific isozyme at GOT_4. There was very little similarity among the species for the three isozymes put together. No *Cucumis* species of African origin seem to have contributed to the evolution of *C. melo*.

Key words: Isoenzymes, *Cucumis* spp. percentage similarity.

Isoenzyme variation in 13 species of *Cucumis*, including *C. melo* (x = 12) and *C. sativus* (x = 7), was studied for three enzyme systems to bring out the affinities if any, among the members of the genus *Cucumis*.

MATERIALS AND METHODS

The *Cucumis* species compared in this study are listed below along with the origin/source of the materials.

Polyacrylamide gel electrophoresis using vertical slab gel was carried out. Table 1 provides the details of the enzymes assayed.

The loci were represented by a numerical subscript eg. GOT_1, PRX_2, etc. The numbering was done in the ascending order from the fastest to the slowest moving anodal loci.

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No. in the figs.	Species/variety	Source
8	*Cucumis melo* L.	IARI, New Delhi
33	*C. anguria* L. GBNR 1970	Wageningen, Netherland
34	*C. anguria* var. *longipes* (Hook. f.) Meeuse GBNR 1735	"
37	*C. africanus* L.F. GBNR 1984	"
38	*C. figarei* Naud. GBNR 1804	"
40	*C. zeyheri* Sond. GBNR 1053 (tetraploid)	"
41	*C. meeusii* Jeffrey GBNR 1800	"
42	*C. melo* var. *agrestis* Naud. GBNR 1987	"
43	*C. ficifolius* A. Rich GBNR 1801	"
35	*C. myriocarpus* Naud. C.73184	South Africa
36	*C. prophetarum* L. IC 155	India
39	*C. zeyheri* Sond. diploid 73252	South Africa
45	*C. sativus* L.	IARI, New Delhi

MEASUREMENT OF SIMILARITY

The degree of electrophoretic similarity among the *Cucumis* species was calculated by pairwise comparison of the genotypes using the method of Sokal and Sneath [1]. Similarity index (S.I.) was calculated by the following formula.

$$S.I. = \frac{\text{No. of homologous bands}}{\text{No. of homologous bands + nonhomologous bands}} \times 100$$

The average of the similarity indices for all the enzymes gave average similarity index for pairwise comparisons.

RESULTS

In the peroxidase zymogram, all the taxa with x = 12 possess the fastest moving anodal band PRX_1. This was absent in *Cucumis sativus* (x = 7). At PRX_2 a cluster of bands was found in all the species, but very few isozymes were common among the species. At PRX_3 and PRX_4 also, there was no similarity. On the whole, there was very little similarity among the species for the peroxidase zymogram (Table 2, Fig. 1).

Table 1. Enzymes assayed in *Cucumis* species

Enzyme	Sample	Gel concentration	Gel buffer	Electrode buffer	Staining technique
Peroxidase (PRX)	Root & hypocotyl region of 4–5 weeks old seedlings	7% acrylamide	Tris-chloride pH 9.0	Tris-glycine pH 8.3	Adopted from Conklin & Smith [8]
Esterase (EST)	3-4 days old seedlings	"	"	"	Shaw & Koen [9]
Glutamate oxalo acetate transaminase (GOT)	"	9.5% acrylamide	"	"	"
Glutamate dehydrogenase (GDH)	"	8% acrylamide	Tris-chloride pH 8.0	"	"

In the GOT system (Fig. 2), on the other hand, all the species shared a common band at GOT_4 under the conditions of GOT analysis. This isozyme was present in *Cucumis sativus* also and absent in other genera like *Praecitrullus* and *Citrullus*. The two *anguria* accessions and *C. prophetarum* had GOT_3 isozyme with similar mobility as that of *C. melo*. Apart from *C. prophetarum*, all the wild species of *Cucumis* shared a common band at GOT_2. *C. prophetarum* had GOT zymogram similar to that of *C. melo*. The two *anguria* accessions, diploid (73252) and tetraploid (GBNR 1053) *C. zeyheri*, *C. africanus*, and *C. figarei* have an identical band at GOT_1. The two *anguria* accessions did not differ in their banding pattern. Thus there was good degree of similarity among the *Cucumis* spp. for the GOT zymogram (Table 2).

In the GDH system (Fig. 3), it was difficult to group the isozymes of different *Cucumis* species under a few loci. The percentage of similarity among the *Cucumis* species for the GDH zymogram is given in Table 2.

Average similarity among the species for the three enzymes is listed in Table 3. As can be seen, none of the species showed appreciable similarity with any other species. The percentage of similarity among the species varied from 8 to 36. *C. melo* and *C. prophetarum* shared 33% common bands between them. The highest S.I. (36%) was between the two *zeyheri* collections. The two *anguria* accessions had 33% S.I. *C. anguria* var. *longipes* had 33% S.I. with *C. melo* also.

C. melo and *C. prophetarum* showed the lowest level of similarity with the remaining species. The two *anguria* accessions showed relatively high level of similarity with all the *Cucumis* species studied.

DISCUSSION

The peroxidase zymogram gave a very complicated picture, having a large number of isozymes with varying mobility in each species (Fig. 1). Even though the banding pattern

Table 2. Percentage of similarity among *Cucumis* species for peroxidase, GOT and GDH zymograms

Enzyme	S. I. of different species*											
	8	33	34	35	36	37	38	39	40	41	42	43
Peroxidase	—											
GOT	100											
GDH	—											
Peroxidase	0	—										
GOT	50.0	100										
GDH	0	—										
Peroxidase	0	0	—									
GOT	50.0	100.0	100									
GDH	50.0	0	—									
Peroxidase	0	0	0									
GOT	28.6	57.1	57.1	100								
GDH	22.2	0	0	—								
Peroxidase	0	0	0	0								
GOT	100	50.0	50.0	28.6	100							
GDH	0	0	0	0	—							
Peroxidase	11.8	0	0	0	23.5	—						
GOT	25.0	75.0	75.0	57.1	25.0	100						
GDH	0	0	0	0	0	—						
Peroxidase	0	0	0	0	0	0	—					
GOT	28.6	85.7	85.7	57.1	28.6	85.7	100					
GDH	0	0	0	0	0	0	—					
Peroxidase	0	0	16.7	0	0	0	0	—				
GOT	28.6	75.0	75.0	57.1	25.0	75.0	85.7	100				
GDH	0	0	0	0	0	0	0	—				
Peroxidase	0	0	26.7	0	0	0	0	0	—			
GOT	28.6	75.0	75.0	57.0	25.0	75.0	85.7	75.0	100			
GDH	0	0	0	0	0	0	0	33.3	—			
Peroxidase	0	0	13.3	0	0	0	0	0	0	—		
GOT	28.6	57.1	57.1	66.7	28.6	57.1	66.7	57.1	57.1	100		
GDH	0	0	0	0	0	0	0	0	0	—		
Peroxidase	0	0	0	0	0	0	0	0	0	0	—	
GOT	28.6	57.1	57.1	66.7	28.6	57.1	66.7	57.1	57.1	66.7	100	
GDH	0	0	0	0	0	0	0	0	0	0	—	
Peroxidase	0	0	0	0	0	0	0	0	0	0	0	—
GOT	28.6	57.1	57.1	66.7	28.6	57.1	66.7	57.1	57.1	66.7	66.7	100
GDH	0	0	0	0	0	0	0	0	0	0	0	—

*S. Nos. of species corresponding to those used in figures (cf. *Materials and Methods*).

was similar, very few isozymes showed identical mobility among the species. Each species had its own individual zymogram. These results are contradictory to Dane's [2] findings, who observed a high degree of similarity among the *Cucumis* spp. for peroxidase zymogram.

Table 3. Average S.I. among *Cucumis* species for peroxidase, GOT and GDH

Species*	8	33	34	35	36	37	38	39	40	41	42	43
8	100											
33	17.0	100										
34	33.0	33.0	100									
35	17.0	19.0	19.0	100								
36	33.0	17.0	17.0	10.0	100							
37	12.25	25.0	25.0	19.0	16.0	100						
38	10.0	29.0	29.0	19.0	10.0	29.0	100					
39	8.0	25.0	31.0	19.0	8.0	25.0	29.0	100				
40	8.0	25.0	34.0	19.0	8.0	25.0	29.0	36.0	100			
41	10.0	19.0	23.0	22.0	10.0	19.0	22.0	19.0	19.0	100		
42	10.0	19.0	19.0	22.0	10.0	19.0	22.0	19.0	19.0	22.0	100	
43	10.0	19.0	19.0	22.0	10.0	19.0	22.0	19.0	19.0	22.0	22.0	100

*Cf. note to Table 2.

On the other hand, Esquinas-Alcazar [3] reported seven different clusters in the species he studied. The two different clusters observed in the cross-compatible species were found to be different from *C. melo*. Similarly, different species showed more differences than similarity in the GDH zymogram also.

In respect of GOT, all the *Cucumis* species studied showed an identical band at GOT_4 (Fig. 2). This band was present in *C. sativus* also, but was absent in other genera like *Citrullus* and *Praecitrullus*. Thus, this isozyme could be a characteristic of the genus *Cucumis*. While all the African wild species (x = 12) showed an identical band at GOT_2, the Indian collection of *C. prophetarum* (x = 12) had an isozyme similar to *C. melo*. The two *anguria* accessions studied had an isozyme at GOT_2 with similar mobility to that of *C. melo* and *C. prophetarum*. The *anguria* collections, diploid and tetraploid *C. zeyheri*, *C. figarei* and *C. africanus* had a similar isozyme at GOT_1. *C. prophetarum* had an identical GOT zymogram with *C. melo*. The two *anguria* accessions also had similar banding pattern. These results are in agreement with the findings of Dane and Esquinas-Alcazar [3] who also found high degree of similarity among the species for GOT.

Summing up the results on isozyme variation in *Cucumis* species, we can conclude that the different species possess their own individual zymogram. There was very little similarity among the species for the three enzyme systems put together. However, there was some similarity among the species, including *C. sativus*, especially for the GOT zymogram, which confirms that all these taxa belong to the same genus, *Cucumis*.

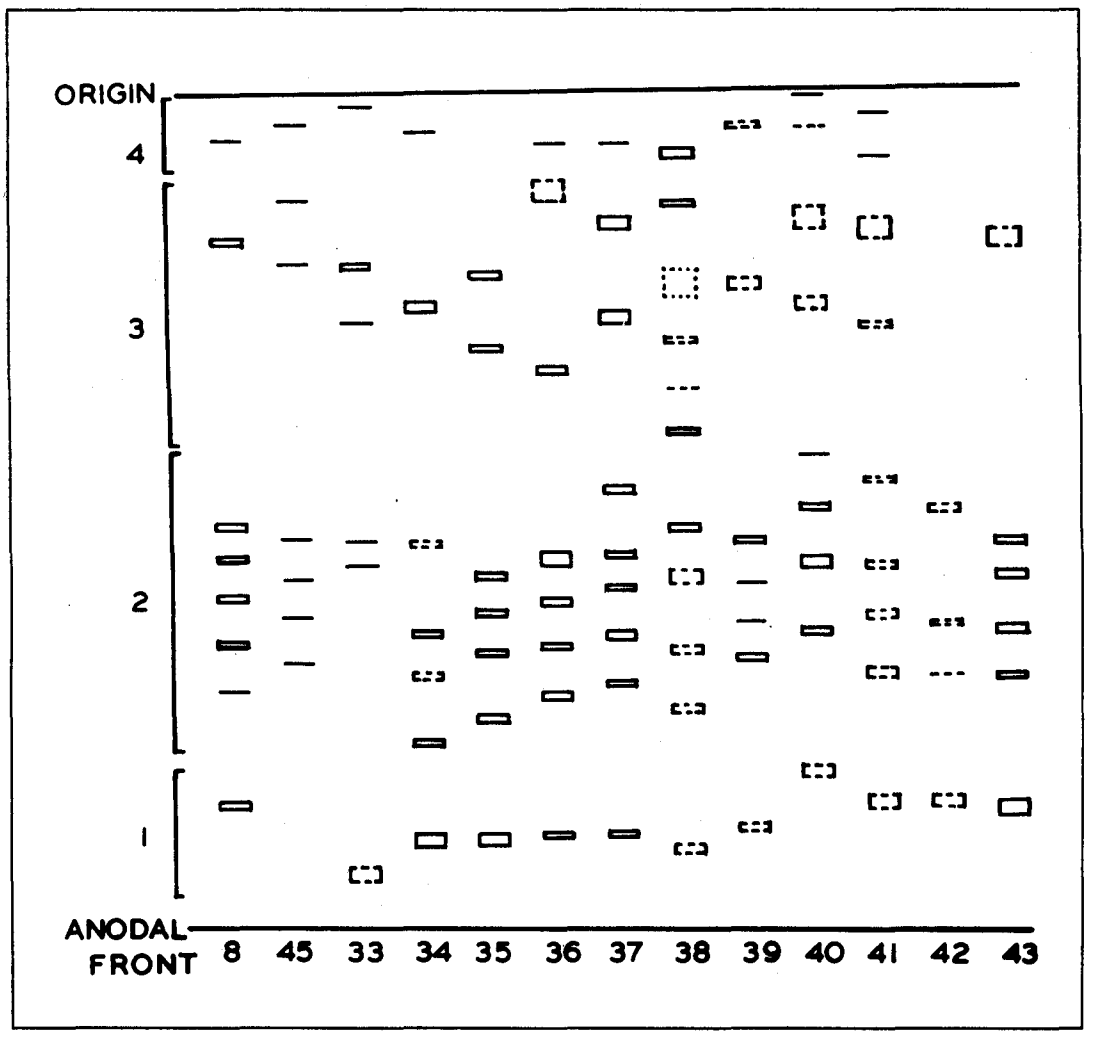

Fig. 1. Peroxidase isozyme pattern of *Cucumis* spp.

On an average, the two *anguria* accessions, *C. anguria* and *C. anguria* var. *longipes*, showed more similarity with other species than any other of the remaining species. On the other hand, *C. melo* and *C. prophetarum* shared minimum similarity with other species. However, they had high S.I. (33%) between them.

C. prophetarum from India is reported to have high degree of cytological homology with *C. melo* [4]. Dane [2] concluded that *C. prophetarum* from India may probably be *C. melo* and appears to be misclassified. In the present study, although *C. prophetarum* has shown an

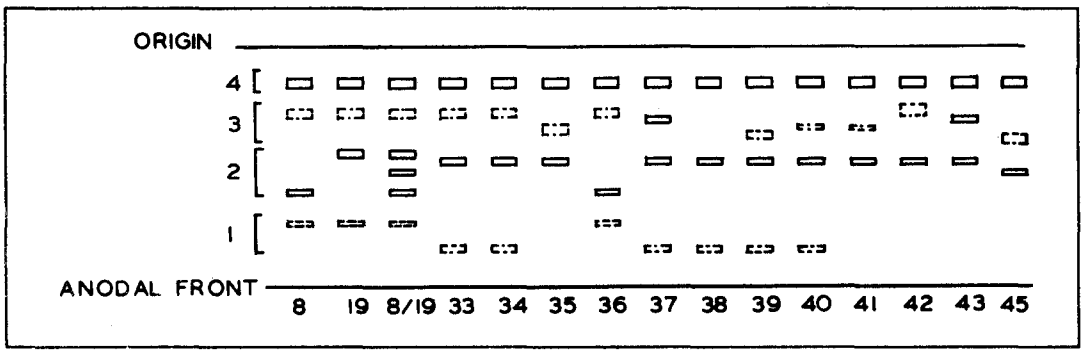

Fig. 2. Variation in GOT zymogram of *Cucumis* spp.

identical GOT zymogram with *C. melo*, the peroxidase and GDH zymograms of the two species were different, thus, justifying their inclusion under separate taxa.

The results of the present study are contradictory to the findings of Dane [2], who found a large degree of similarity among the *Cucumis* species for the peroxidase zymogram. Our results also do not agree with those of Esquinas-Alcazar [3], who classified *Cucumis* species into four groups based on Nei's genetic distance between them.

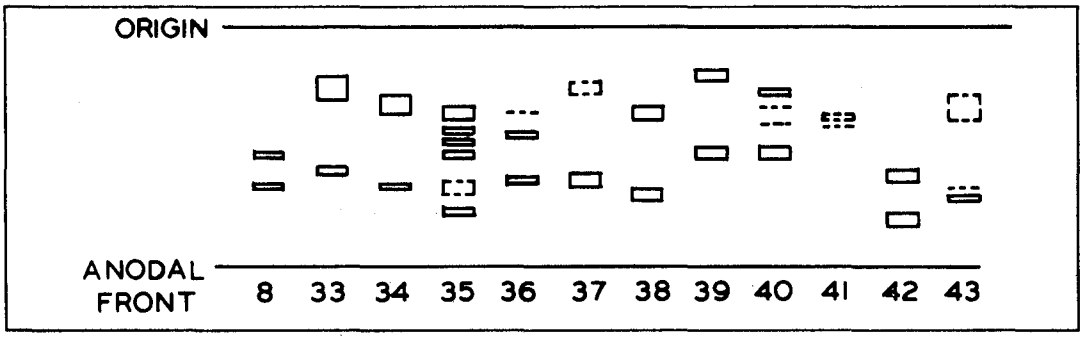

Fig. 3. Variation of GDH isozymes in *Cucumis* spp.

These observations lend support to the view that no *Cucumis* species (with x = 12) of African origin contributed to, or is involved in, the evolution of *C. melo* or the common muskmelon. Isozyme studies do corroborate the observations of a certain degree of remoteness of *C. melo* from the other 12-chromosome species found in the crossability studies [5–7]. In other words, the diversification within *C. melo* and apparent morphological variation within the taxon had no relationship within *C. melo* and apparent large morphological variation within the taxon had no relationship with the other 12-chromosome species of African origin.

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