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Inheritance and linkage relationships of isozyme loci in cucumber (*Cucumis sativus* L.)

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Summary. Genetic analyses were conducted among 18 provisionary isozyme loci in *Cucumis sativus* L. Fourteen loci demonstrated simple Mendelian inheritance while observed variation at four loci (*Gpi2*, *Gr2*, *Pgm3*, *Skdh2*) was determined not to have a predictable genetic basis. Joint segregation analyses among the 14 genetically predictable polymorphic loci resulted in the assignment of 12 loci to four linkage groups. Linkage groups contain the following loci: (1) *Gr1*, *Pgm1*, *Idh*, *Pgd1*; (2) *Pep-pap*, *Mdh2*, *Mdh3*, *Gpi1*; (3) *Pep-la*, *Per4*; (4) *Pgd2*, *G2dh*. *Mpi2* and *Mdh1* segregated independently. Recombination fractions for linked loci ranged between 0.051 (*Pgm1-Idh*) to 0.385 (*Pep-la-Per4*). Some practical applications of isozyme marker loci for cucumer improvement are discussed.

Key words: Cucumber – Gene mapping – Isozymes – Segregation – Starch-gel electrophoresis

Introduction

Biochemical genetic markers have proven to be useful tools in the plant sciences. Although much of the current research is focusing on restriction fragment length polymorphisms, isozymes remain extremely useful and should not be overlooked as valuable molecular markers. Allozymes have been used for confirmation of hybridity (e.g., Lo Schiavo et al. 1983), cultivar identification (e.g., Nielsen 1985), detection of somatic variation (e.g., Lassner and Orton 1983), estimation of mating systems (e.g., Ritland 1983) and gene dosage effects (e.g., Birchler 1983), genome identification (e.g., Pierce and Brewbaker 1973), measurement of genetic variation (e.g., Brown and Weir 1983), characterization of taxonomic relationships (e.g., Crawford 1983), purity testing of commercial seedlots (e.g., Arús 1983), and varietal patenting (e.g., Bailey 1983). Allozymes have also been used as genetic markers in linkage studies to facilitate, the construction of genomic maps (Goodman et al. 1980, Navot and Zamir 1986, Tanksley 1983a; van Heemstra et al. 1991; Weeden and Lamb 1987). Genomic maps can be used to aid in the introgression of chromosome segments into various genetic backgrounds (Rick and Khush 1969; Quiros et al. 1987), to localize genes for addition to existing maps (Aicher and Sounders 1990; Kiang and Bult 1991; Patterson 1982), to determine homologies among divergent taxa (Tanksley 1983b; Walters et al. 1991), and as a tool for increasing selection efficiency (Abler et al. 1991; Shenoy et al. 1990; Tanksley and Rick 1980).

An important practical use of linkage information lies in its potential for increasing selection efficiency. Linkages have been detected between isozyme loci and loci controlling morphological and economically important traits (e.g., O'Brien 1990). When these associations are tight (1–2 cM) allozyme analysis can be used effectively for increasing selection efficiency. For instance, the association between an acid phosphatase allozyme and nematode resistance in tomato has proven to be an important selection tool (Rick and Fobes 1974). Associations also have been demonstrated between isozyme loci and quantitative trait loci such as yield (Frei et al. 1986) and cold tolerance (Guse et al. 1988; Vallejos and Tanksley 1983). In some instances allozyme analysis has proven useful for increasing gain from selection of metric traits when compared to more conventional procedures (Frei et al. 1986).

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Limited information exists regarding linkage relationships in cucumber (Fanourakis and Simon 1987a, b; Pierce and Wehner 1990). Because cucumber is a diploid species with seven chromosomes and approximately 105 described genes (Pierce and Wehner 1990), many linkages might be expected. There are 5,460 pairwise combinations that could be tested among the 105 described genes. Recombination fractions have been reported for only 29 (0.5%) of these pairwise combinations (Knerr 1990). Linkages of undetermined magnitude have been published for an additional 37 pairwise combinations and independence has been reported for 113 pairwise combinations (Pierce and Wehner 1990). Therefore, combined linkage information is available for only 179 (3.3%) of the 5,460 possible pairwise combinations. This is largely due to the lack of multiple gene heterallelic marker stocks established in common background inbreds.

Many single gene morphological traits are undesirable as genetic markers due to their phenotypic effects. Allozymes, unlike many morphological traits conditioned by single genes, are less susceptible to environmental modification (Tanksley and Orten 1983), and they lack pleiotropic and epistatic interactions (Brown and Weir 1983; Tanksley 1983b; Weeden and Wendel 1989). An extensive cucumber allozyme linkage map in combination with lines possessing multiple variant alleles would allow for efficient linkage evaluations between several allozymes and single-gene morphological traits. If sufficient numbers of allozyme markers were available, then indirect selection for quantitative trait loci may be possible (Tanksley et al. 1981). Therefore, cucumber strains possessing multiple allozyme markers were developed to determine the inheritance and linkage relationships among enzyme coding loci.

Materials and methods

Plant materials and family construction

Seven hundred and fifty-sevent plant introductions (PIs) from the U.S. cucumber (*Cucumis sativus* L.) germ plasm collection were originally assayed for variation at biochemical loci thought to possess heritable genetic variation using horizontal starch gel electrophoresis (Knerr et al. 1989). Preparation of sample material, electrophoresis, and allozyme nomenclature follows that of Staub et al. (1985): enzymes are designated by uppercase abbreviations; loci coding for enzymes (uppercase) are designated by the first letter being uppercase and the rest lowercase. If an enzyme is coded by multiple loci, these are designated by numerals and are numbered from most cathodal to most anodal. Alleles of a given locus are numbered from most cathodal to most anodal and enclosed in parentheses. The mobility of the most common allele of a locus was designated 100, and all other alleles were assigned a value based on the mobility (mm) of their homomeric protein product relative to that of allele 100. For example, an allele at locus 1 of GPI which had a mobility 2 mm less than the most common allele was assigned the designation *Gpi1(1)*-98.

Intraspecific crosses were made between accessions possessing dissimilar homozygous alleles for given loci to produce F_1 individuals. Table 1 lists sources of less common alleles based on a previous study by Knerr et al. (1989). If only heterozygotes were identified with an accession during screening, they were selfed and crossed to homozygous individuals to test for segregation and to recover homozygotes. These F_1 individuals were used to produce F_2 and backcross (BC) families. BC families were produced to aid in the construction of lines possessing multiple allozyme polymorphisms for linkage analyses.

Assessment of unpredicted electromorphs

Electrophoresis of cross progenies revealed that predicted phenotypes were not observed after staining for GPI (locus 2),GR (locus 2), PGM (locus 3), and SKDH (locus 2). Therefore, a series of experiments was conducted to determine the basis for these observations. For GPI, 10 seeds of four PIs [two (PIs 175690, 181942) presumed to be heterozygous (12) and two (PIs 267749, 354952) homozygous (11)] were dark germinated in 100-mm filter-paper-lined petri dishes containing 7 ml of distilled water for 5 days at 23 °C.

One cotyledon from each germinated seed was then removed and analyzed for variation at GPI locus 2. Concurrently, seeds of each PI were germinated in vermiculite in a greenhouse (16-h photoperiod supplemented by cool-white fluorescent lights providing about 100 μmol s^{-1} m^{-2}; 17–35 °C minimum/maximum temperatures). One cotyledon was removed from each germinated seed and examined electrophoretically when the hypocotyl emerged, but cotyledons had not expanded (5 days). The remaining 5-day-old dark-germinated sedlings were also exposed to light by placing them in the greenhouse with seedlings growing in vermiculite. Seedlings were sampled in all growing environments after 48 and 96 hours, respectively, and cotyledonary extracts were assayed electrophoretically. Additionally, leaf tissue of plants with 10–14 leaves was assayed for variation at *Gpi2*.

Electromorphs not predicted for *Gr2*, *Pgm3*, and *Skdh2* were evaluated by crossing putative homozygous (11×22[1]) plants for *Gr2* and putative homozygous and heterozygous (11×12) plants for *Pgm3* and *Skdh2*. Putative *Pgm3* and *Skdh2* heterozygotes were also selfed. Cotyledonary tissue of resulting progeny was then evaluated for allozyme variation.

Inheritance, linkage analysis, and gene order determination

Data obtained from F_2 and BC families were analyzed by chi-square goodness-of-fit for conformance to expected 1:2:1 or 1:1 segregation ratios. A chi-square test for heterogeneity also was conducted on pooled multiple family data.

Contingency chi-square analyses were performed to test for non-random joint segregation among all pairwise combinations of loci. Recombination fractions and their standard errors were calculated using maximum likelihood procedures (Hutchinson 1929; Mather 1951, Bailey 1961) for all pairs of loci that deviated from independent segregation ($P < 0.05$). Data from F_2 and BC families were tested for heterogeneity and pooled using a chi-square test to estimate linkage. These analyses were facilitated by the use of the LINKAGE-1 computer program (Suiter et al. 1983). All combinations were tested in a minimum of three F_2 families and two BC families with the following exceptions: (1) pairwise combinations of loci involving *Gr1* were tested in a minimum of two BC families, and (2) joint segregation of *Pep-la*

[1] 11 represents a plant with two copies of the slower allele (homozygous), 22 a plant with two copies of the faster allele (homozygous), and 12 a plant with one copy of each allele (heterozygous)

Table 1. Sources of putative allozyme variation for inheritance and linkage studies in cucumber (*Cucumis sativus* L.)[a]

Allozyme	Plant introduction	Source country
Gpi1(1)	176524	Turkey
	200815	Burma
	249561	France
	422192	Hungary
	432854	People's Republic of China
	436608	People's Republic of China
Gpi2(2)	175690	Turkey
	177359	Turkey
	177364	Iraq
	181942	Syria
	391569	China
Gr1(3)	109275	Turkey
Gr2(1)	179676	India
	183056	India
	183127	India
G2dh(1)	285606	Poland
Idh(1)	215589	India
	183967	India
Mdh1(2)	171613	Turkey
	209064	United States
	326594	Hungary
Mdh2(1)	174164	Turkey
	185690	Turkey
	357835	Yugoslavia
	419214	Hong Kong
Mdh3(2)	255236	Netherlands
	267942	Japan
	432854	People's Republic of China
	432887	People's Republic of China
Mpi2(2)	109275	Turkey
	175692	Turkey
	200815	Burma
	209064	United States
	263049	Soviet Union
	354952	Denmark
Pep-la(4)	169380	Turkey
	175692	Turkey
	263049	Soviet Union
	289698	Australia
	354952	Denmark
Pep-pap(1)	163213	India
	188749	Egypt
	432861	People's Republic of China
Per4(2)	215589	India
Pgm1(2)	171613	Turkey
	177364	Iraq
	188749	Egypt
	263049	Soviet Union
	264229	France
	285606	Poland
	289698	Australia
	354952	Denmark
Pgm3(2)	174170	Turkey
	178888	Turkey
	209064	United States
	267741	Japan
	288992	Hungary
Pgd1(1)	169380	Turkey
	175692	Turkey
	222782	Iran
Pgd2(2)	171613	Turkey
	177364	Iraq
	188749	Egypt
	263049	Soviet Union
	285606	Poland
	289698	Australia
	354952	Denmark
	419214	Hong Kong
	432858	People's Republic of China
Skdh2(2)	174177	Turkey
	178888	Turkey
	188749	Egypt
	209064	United States
	306180	Poland

[a] Sources are only given for the less common allele of a locus used in this study based on a previous study by Knerr et al. (1989)

with *Idh*, *Per4*, and *Pgd1* and *Per4* with *Pgd1* were tested in three F_2 families and one BC family. In cases where more than one segregating family was available, data were pooled to calculate a combined linkage estimate.

Where appropriate, gene order was determined using recombination fractions. In certain cases (*Gpi1*, *Mdh2*, *Mdh3* and *Idh*, *Pgm1*, *Pgd1*), triple backcross segregations were also analyzed to confirm gene order (Bailey 1961).

Results and discussion

Non-heritable variation

Observed polymorphisms at some isozyme loci did not adhere to expected Mendelian segregation ratios. The variation observed initially at *Gpi2* was probably due to developmental changes (Knerr et al. 1989). Triple-banded isozyme patterns suggested the presence of dimeric enzyme expression in some accessions. However, upon selfing of these putative heterozygotes, resulting progeny possessed only one enzyme band. Seedlings with fully expanded cotyledons which emerged from vermiculite (5 days after planting) displayed a single band at *Gpi2*. In contrast, dark-germinated and unexpanded cotyledonary tissue produced three bands (relative mobility in mm = 100, 101, 102) in the *Gpi2* gel region for all four PIs. Electrophoresis 48 h later revealed the disappearance of the most anodal band (102); only two bands (100 and 101) were present. Electrophoretic evaluation 96 h later demonstrated the presence of only the most cathodal band (100). This band was observed in extracts from mature plants and therefore remains active through-

out plant development. Tanksley and Rick (1980) observed similar phenomena for GPI in tomato.

The disappearance of isozymes after exposure to light has been documented for two cytosolic MDH isozymes in cucumber cotyledons (Liu and Huang 1976). The subcellular localization of *Gpi2* is not known for cucumber, but in other plant species the chloroplast-specific GPI migrates faster than the cytosolic fraction (Weeden and Wendel 1989). In addition to more anodal migration (Ireland and Dennis 1980), plastid isozymes also generally possess lower variation than their cytosolic counterparts (Gottlieb and Weeden 1981). Given that *Gpi1* variation is low (Knerr et al. 1989) and assuming that *GPi2* is a plastid isozyme, the observations may help to explain the lack of observed variation at *Gpi2*.

Variation for *Gr2* was not considered heritable after the crossing of presumed homozygotes (11 × 22) failed to result in the production of heterozygotes (12). In addition, selfing of homozygotes occasionally resulted in progeny that produced bands with slightly different mobilities. Likewise, crossing putative homozygotes and heterozygotes (11 × 12), and selfing heterozygotes failed to result in segregating progeny at *Pgm3*. Observable variation may be the result of in vivo or in vitro post-translational modifications that can reduce the activity or modify the structure of the enzyme (Moos 1982). These changes include deamination, acetylation, partial proteolysis, nonproteolytic alterations, additions of foreign groups, and disulfide/hydrogen bridging (Dreyfus et al. 1978; Harris and Hopkinson 1976). Staub et al. (1985) noted such modifications in cucumber enzymes maintained at varying storage regimes.

An alternate explanation may be varying levels of co-factor saturation that can affect band mobility (Harris and Hopkinson 1976). For instance, different electrophoretic components may be observed for enzymes which require NAD or NADP as coenzymes according to the degree to which the enzyme is saturated with the coenzyme. If saturation is partial, different bands may represent the same isozyme, to which 0, 1, 2, or more coenzyme molecules are bound.

Progeny resulting from matings of *Skdh2* variants did not segregate. Additional bands appeared on all gels after a varying length of time. These bands were anodal to the initial band observed when staining for SKDH. After consistent variable expression was observed among individuals on a single gel, *Skdh2* variation was determined to be an artifact (May 1980). Artifact bands appear to be related to the activity of the initial band and are the consequence of post-translational modifications (Harris and Hopkinson 1976).

Single factor segregation

Fourteen of the 18 loci identified by Knerr et al. (1989) demonstrated agreement with the expected 1:2:1 and 1:1 segregation ratios in the F_2 and BC generations, respectively (Table 2). Chi-square analyses for heterogeneity were non-significant, indicating homogeneity across families for all loci.

Table 2. Observed single-locus numbers in F_2 and backcross (BC) families and deviation χ^2 value among F_2 and BC families for 14 isozyme loci in cucumber (*Cucumis sativus* L.)[a]

Isozyme	Genotypes					χ^2	
	F_2			BC		F_2	BC
	11	12	22	11	12		
Gpi1	141	257	151	267	279	2.67 (4.75)	0.45 (3.80)
Gr1	–	–	–	341	340	– (–)	0.00 (2.68)
G2dh	164	304	151	272	290	0.74 (2.89)	0.56 (4.63)
Idh	137	272	140	278	305	0.08 (5.56)	1.25 (4.86)
Mdh1	157	271	153	306	319	2.67 (4.25)	0.27 (3.99)
Mdh2	145	301	125	164	188	2.20 (4.80)	1.64 (2.51)
Mdh3	179	321	163	235	231	1.44 (4.16)	0.03 (4.16)
Mpi2	159	294	157	319	331	0.81 (4.75)	0.22 (1.93)
Pep-la	155	261	143	272	275	2.90 (6.95)	0.02 (5.15)
Pep-pap	169	367	182	286	261	0.83 (3.86)	1.06 (2.29)
Per4	170	321	162	258	258	0.38 (5.72)	0.00 (5.72)
Pgm1	131	277	144	257	267	0.62 (4.24)	0.19 (7.08)
Pgd-1	153	298	150	246	231	0.07 (4.87)	0.47 (4.73)
Pgd2	200	377	160	282	325	4.73 (2.26)	3.19 (1.73)

[a] Heterogeneity χ^2 values are given in parentheses. All χ^2 values have a corresponding $P > 0.05$. Degrees of freedom for $F_2 = 2$, BC = 1; heterogeneity = (number of families − 1). A total of six F_2 and six BC families were assayed for each locus except for *Gr1* and *Pep-la*. No F_2 families were available for *Gr1* and only 5 BC families were available for *Pep-la*

Descriptions of polymorphic loci

G2DH, PGM, and PER are monomeric enzymes and possess one variable locus with two alleles each. G2DH is unique in that two co-segregating bands occur in conjunction with heritable variation that gives the appearance of three-banded homozygotes (Fig. 1). Two co-segregating bands appear just cathodal to the initial band for each allele [*w*, *x* and *y*, *z*, respectively (Fig. 1)]. The most cathodal co-segregating band (*y*) of the most anodal allele (2) overlaps with the most cathodal allele (1), resulting in a five-banded heterozygote (Fig. 1). MPI and PEP-LA are monomeric enzymes that possess one variable locus with four and five alleles, respectively.

IDH, PEP-PAP, and PGD are dimeric and they possess one, one, and two diallelic loci, respectively. GPI is dimeric and has one variable locus with three codominant alleles. MDH is also dimeric and possesses three variable loci each with two codominant alleles. MDH is probably the most studied of the polymorphic enzymes in cucumber as cellular compartmentalization has been identified for these isozymes. When interpreting the work of Liu and Huang (1976), *Mdh1*, *-2*, and *-3* hypothetically

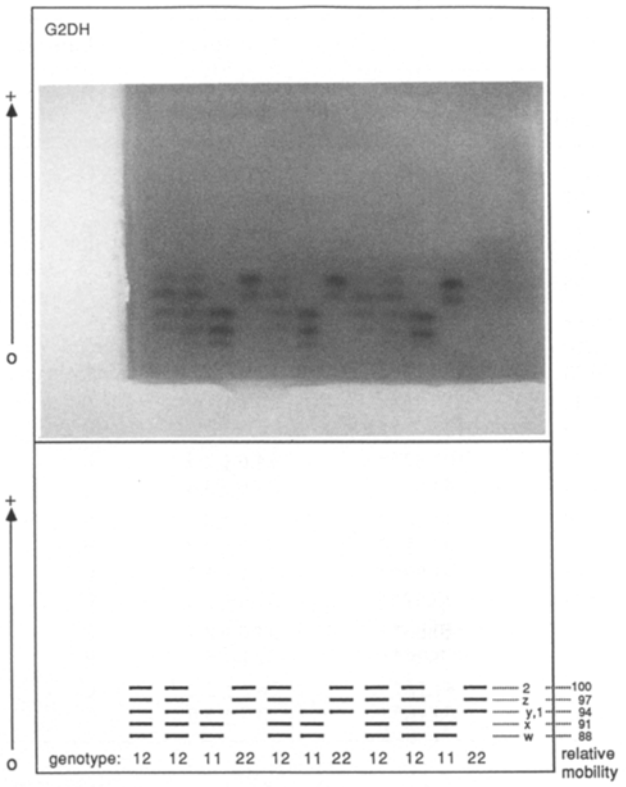

Fig. 1. Photograph and diagrammatic representation showing variation at glycerate dehydrogenase (G2DH; monomeric). Products of each allele [*1* (94) and *2* (100)] exhibit two co-segregating bands (*w*, *x* and *y*, *z*). The most cathodal co-segregating band (*y*) of the most anodal allelic product (*2*) overlaps with the most cathodal allelic product (*1*), resulting in a five-banded heterozygote

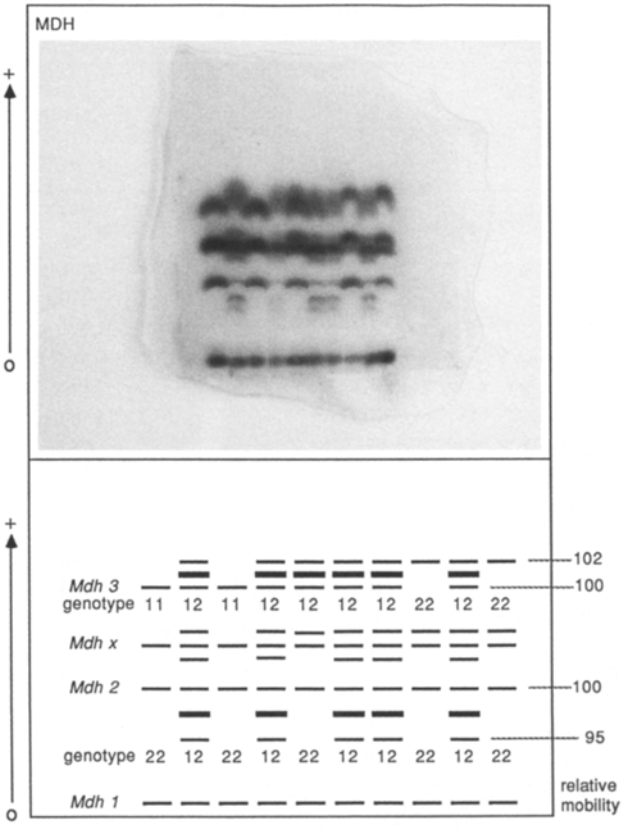

Fig. 2. Photograph and diagrammatic representation showing variation at locus 2 and locus 3 for malate dehydrogenase (MDH; dimeric) and interlocus dimerization between these loci. *Mdh-x* may overlap with another non-variable MDH locus as one *Mdh-x* band is consistent regardless of variation at *Mdh2* or *Mdh3*

code for microbody, mitochondrial, and cytosolic isozymes, respectively. Liu and Huang (1976) also reported two additional cytosolic MDH isozymes present only in dark-grown cotyledons. In our studies, an interlocus heterodimer, designated as Mdh-x, was observed between the products of *Mdh2* and *Mdh3* (Fig. 2). This MDH interlocus dimerization phenomena has been observed in other species as well (Goodman et al. 1980). GR is a tetrameric enzyme with three codominant alleles present at one variable locus.

Joint segregation

Since the observed variation at *Gpi2*, *Gr2*, *Pgm3*, and *Skdh2* did not have predictable genetic bases, these loci were omitted from linkage studies. Data from segregating F_2 and BC families were used to test the joint segregation of 14 loci (91 pair-wise combinations). Eleven pairs of loci demonstrated linkage in all of the populations tested (Table 3).

The 12 loci demonstrating linkage in segregating families were placed into four linkage groups (*Gr1*, *Pgm1*, *Idh*, *Pgd1*; *Pep-pap*, *Mdh2*, *Mdh3*, *Gpi1*; *Pep-la*, *Per4*; *Pgd2*, *G2dh*) (Table 3, Fig. 3). Analysis indicated that *Mdh1* and *Mpi2* could not be assigned to a linkage group. Heterogeneity was minimal [$P > 0.05$ in all cases except joint segregation between *Pep-la* and *Per-4* in F_2 families for the 80 pairs of loci that segregated independently (data not presented)].

In two cases, triple backcross data were analyzed to confirm gene order (Table 4). The data from these families supported the suspected gene order determined for linkage groups 1 and 2 from joint segregation data. These families also allowed for an estimation of chiasma interference that was low in both cases (0.07 to 0.16), indicating that incidence of crossing-over in one region did not affect crossing-over in a neighboring region for these groups of loci. Chiasma interference also has been reported to be low in cucumber for other loci (Fanourakis and Simon 1987a).

Table 3. Deviation chi-square values, recombination values, and linkage phase for linked isozyme loci in cucumber (*Cucumis sativus* L.) derived from F_2 and BC families

Isozyme pair	Gen.	Number of families	Number of plants	χ^2		Crossover % ± SE	Linkage phase[a]
				Hetero-geneity	Linkage		
Gpi1, Mdh2	F_2	3	253	1.32	54.62**	31.6±2.6	C
	BC	3	248	0.01	35.00**	31.6±2.9	C
Gpi1, Mdh3	F_2	3	298	4.83	114.04**	23.3±2.0	R
	BC	4	230	0.62	69.75**	24.4±2.8	R
Gr1, Idh	BC	2	221	0.05	64.18**	23.1±2.8	R
Gr1, Pgm1	BC	3	315	1.15	125.29**	18.4±2.2	C
G2dh, Pgd2	F_2	3	328	2.10	157.99**	21.6±1.9	C
	BC	3	304	0.33	66.12**	27.0±2.5	C
Idh, Pgm1	F_2	3	254	2.40	375.55**	5.1±1.0	R
	BC	3	225	0.43	187.01**	4.4±1.4	R
Idh, Pgd1	F_2	3	208	2.31	107.82**	24.6±2.5	R
	BC	2	157	0.04	43.93**	23.6±3.4	R
Mdh2, Mdh3	F_2	3	280	3.90	148.73**	24.2±2.1	R
	BC	2	144	0.72	37.31**	24.5±3.6	R
Mdh2, P-pap	F_2	3	327	1.68	93.99**	29.1±2.2	C
	BC	2	145	0.24	20.57**	31.0±3.8	C
Pep-la, Per4	F_2	3	286	8.24*	40.66**	38.6±2.7	R
	BC	1	92	–	4.24*	39.1±5.1	R
Pgm1, Pgd1	F_2	3	302	3.00	81.56**	26.5±2.2	C
	BC	3	207	0.05	58.10**	23.7±3.0	C

* $P < 0.05$; ** $P < 0.01$

[a] C = coupling, R = repulsion. In this case, the use of the term 'linkage phase' refers to the association of fast-migrating alleles with slow-migrating alleles (R) or slow (fast) alleles with slow (fast) alleles (C). In the case of complete classification linkage phase does not affect the amount of information provided per individual (Allard 1956)

Table 4. Observed number of individuals and the assigned linear order and level of chiasma interference from single families segregating for three isozyme loci in cucumber[a]

Loci	Progeny classes[a]								Gene order	Chiasma interference
	+++	−−−	+−−	−++	++−	−−+	+−+	−+−		
(*Mdh2, Mdh3, Gpi1*)	17	13	5	9	7	7	2	1	*Mdh2, Mdh3, Gpi1*	+0.07
(*Idh, Pgm1, Pgd1*)	37	43	0	1	14	11	1	3	*Pgm1, Idh, Pgd1*	+0.16

[a] Isozyme locus designations were altered for ease of presentation, + = 11 and − = 12

Although high, the incidence of linkage observed might have been predicted given cucumber's low basic chromosome number ($n = 7$), small chromosome size (Ramachandran et al. 1983; Whitaker and Davis 1962), and low cross-over frequency (Fanourakis 1984). In a linkage study utilizing morphological markers Fanourakis and Simon (1987a) placed 13 of 15 loci into three linkage groups.

The linkage map constructed from these data indicates the potential utility of cucumber allozymes as genetic markers. Preliminary data (Staub, unpublished) indicate that associations exist between an isozyme locus and spider mite/thrip resistance. If genetic linkages exist between economically important traits and isozyme loci, then isozymes could be used as markers to increase selection efficiency in cucumber improvement programs.

More allozymic variation might be expected since many loci were polymorphic in low frequencies (<1%) when 16% of the collection was initially screened for variation (Knerr et al. 1989). Therefore, we have initiated further screening of the U.S. cucumber germ plasm collection and adapted U.S. and European cultivars in order to facilitate linkage analyses of allozyme markers and morphological traits. This screening involves 40% of the collection (approximately 800 PIs currently available) using 50 enzyme systems (Staub et al. 1985) not used in the

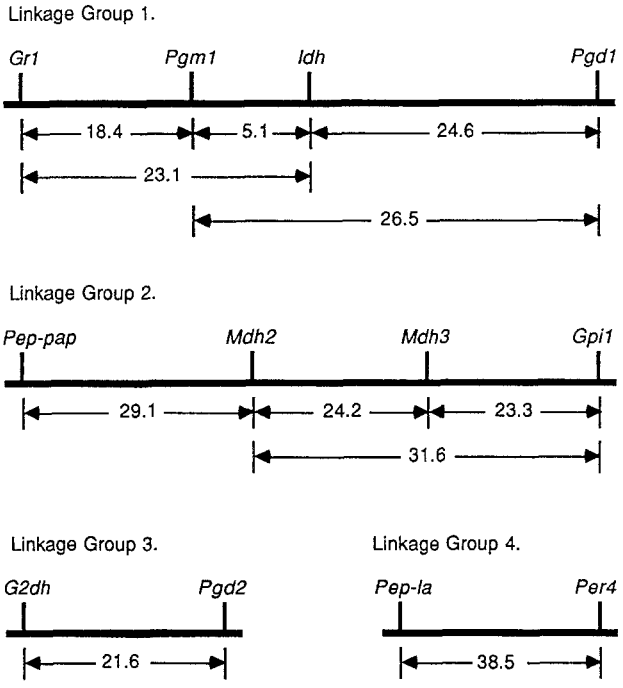

Fig. 3. Linkage map for 12 isozyme loci based on F_2 and BC populations of cucumber (*Cucumis sativus* L.)

initial survey (Knerr et al. 1989). We are also constructing nearly isogenic processing, market and Dutch greenhouse cucumber lines which contain all polymorphic loci. These lines can be used by breeders to develop lines and hybrids with unique arrays of marker loci useful for assessing seed lot purity and/or for establishing varietal identity for plant variety protection.

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