Inheritance and Linkage Relationships of Melon (*Cucumis melo* L.) Isozymes

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ABSTRACT. Nineteen polymorphic and eleven monomorphic isozyme loci were identified in thirteen enzyme systems in a survey of four-hundred melon (*Cucumis melo* L.) accessions. Segregation of allozymes in F₂ and backcross (BC) families for isozyme loci agreed with the expected 1:2:1 and 1:1 segregation ratios ($P < 0.01$). Eleven isozyme loci were linked and were integrated to form a map containing two linkage groups spanning 98 cM with a mean linkage distance of ≈ 9 cM. Linkage groups (A and B) contain the following loci in the order: A *Fdp-2*, *Pgd*, *Pgm*, *Mpi-1*, *Idh*, and *Ac*, and B *Pep-gl*, *Mdh-2*, *Mdh-4*, *Mdh-5*, *Mdh-6*. The remaining eight loci (*Acp-1*, *Acp-4*, *Ak-4*, *Fdp-1*, *Gpi*, *Mpi-2*, *Pep-la*, and *Pep-pap*) segregated independently. The isozyme map constructed in this study provides genomic information for future linkage studies with economically important traits and consensus map construction through map merging.

Genetic markers have wide applicability in *Cucumis* breeding and have been used for cultivar discrimination, seed purity testing, and biosystematic and taxonomic studies (Staub and Meglic, 1993; Staub et al., 1992). Esquinas-Alcazar (1981) used six isozyme systems to examine the genetic diversity of Spanish and U.S. melon (*C. melo* L.) accessions (populations). More recently, Neuhausen (1992) used RFLP markers for cultivar discrimination in melon. DNA sequence variation examined by polymerase chain reaction (PCR) amplification and direct sequencing revealed limited polymorphism among melon cultivars (Shattuck-Eidens et al., 1990). Genetic variation such as that observed in repetitive sequences (satellite DNA) has been used for taxonomic classification of melon (Torres-Ruiz and Hemleben, 1991; Zentgraf et al., 1992).

The increased numbers of traits to be combined into modern melon cultivars (particularly F₁ hybrids) demands techniques where selection for one trait does not preclude, interfere, or confound selection for other traits. Genetic markers have utility for marker-assisted selection (MAS) if marker-trait linkages are relatively tight (< 5 cM) (Staub et al., 1996). Such linkage relationships can be used to increase breeding efficiency by allowing earlier selection and reducing plant population size during selection. Additionally, codominant markers can be used to select indirectly for recessive traits during backcrossing (i.e., without the necessity of selfing).

In melon, MAS could be used to increase selection efficiency for diseases such as powdery mildew and viruses where disease symptoms of various strains are often difficult to interpret or where genotype conformation requires extensive progeny testing. Cur-

rently, breeding efforts in melon are being made to combine resistances to viruses that cause cucumber mosaic (CMV), watermelon mosaic (WMV-2), papaya ringspot (watermelon strain) (PRSV-W), and zucchini yellow mosaic (ZYMV). However, plant inoculation with one of these viruses often interferes with selection for resistance to a second. Likewise, selection for resistance to lettuce infectious yellows and beet pseudo-yellows viruses is labor intensive since plants must be inoculated using their whitefly vectors and symptoms do not become apparent until ≈ 4 to 6 weeks postinoculation. In this case, symptom expression can be confounded by leaf senescence. Similarly, traits of melon that are affected by developmental conditions (e.g., sex expression and vine habit) could be selected more efficiently using genetic markers that are less affected by the environment.

Published information regarding genetic linkages among economically important traits in melon is sparse (McCreight, 1983; Pitrat, 1984 and 1991). Pitrat (1994) summarized linkage information in melon. The thirteen linkage groups consist of one group with five markers, one group with four markers, two groups with three markers, and four groups with two markers. The remaining five groups consist of one marker each. Some of these linkage groups contain economically important genes. For instance, *Fusarium* race 1 resistance is linked to papaya ringspot virus resistance (*Prv*). Resistance to *Fusarium* race 2 (*Fom-2*) is linked to male sterile-2 (*ms-2*) and yellow green foliage (*yg*). Likewise, the gene conditioning powdery mildew resistance (*Pm-x*; from PI 414723) is in the same linkage group as zucchini yellow mosaic virus resistance, andromonoecious (*a*), oval fruit shape (*O*), and halo cotyledons (*h*). The gene order, however, in this group is not known.

A second gene for resistance to powdery mildew (*Pm-w*; from WMR 29) is linked to virus aphid transmission resistance (*Vat*) and flaccida necrosis resistance (*Fn*), and a third gene *Pm-3* is linked to necrotic spot virus resistance (*nsv*). Gene *Pm-3* confers resistance against race 2 of powdery mildew. Recently, Baudracco-Arnas and Pitrat (1996) have constructed a 103-point map in melon using isozymes, restriction fragment-length polymorphisms

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(RFLPs), randomly amplified polymorphic DNA (RADP) markers, and disease resistance markers. This map is the first comprehensive report of the molecular structure of the melon genome. The mapping population segregated for 6-PGD, and one *6-pgd* locus was placed in a linkage group between flanking molecular markers.

Isozyme analysis has been applied to cultivar identification and F_1 hybrid production in melon (Chen et al., 1990; Sujatha et al., 1991; Sujatha and Seshadri, 1991). Molecular markers have not, however, been used in melon improvement programs as a selection tool. Although isozyme-trait associations have not been reported

in melon, isozymes have been found to be closely linked to economically important traits in cucumber (*Cucumis sativus* L.) (Meglic and Staub, 1996). We report here the inheritance and linkage relationships of 19 isozyme loci in melon. Data from this study provides additional genetic information for further work in molecular map construction in melon.

Materials and Methods

GERMPLASM. Four-hundred *C. melo* plant introductions (PIs) were obtained from the U.S. Department of Agriculture (USDA),

Table 1. Melon (*C. melo* L.) plant introductions (PI; U.S. Department of Agriculture, North Central Regional Plant Introduction Station, Ames, Iowa) used in an survey to identify isozyme polymorphisms.

PI	Country	PI	Country	PI	Country	PI	Country	PI	Country
93438	China	123689	India	157081	China	182964	India	255478	S. Korea
93799	China	123821	India	157082	China	183256	Saudi Arabia	255479	S. Korea
93800	China	123822	India	157083	China	183257	Saudi Arabia	255946	Germany
102077	Morocco	123823	India	157084	China	183311	India	255952	United States
109479	Turkey	123824	India	157085	China	185111	Ghana	261778	France
116479	India	124092	India	161375	S. Korea	192939	China	266928	Japan
116915	Afghanistan	124093	India	164187	India	193495	Ethiopia	266929	Japan
116916	Afghanistan	124096	India	164320	India	194057	China	266930	Japan
116917	Afghanistan	124098	India	164418	India	200813	Myanmar	266931	Japan
117158	Turkey	124099	India	164493	India	200814	Myanmar	266932	Japan
117162	Turkey	124100	India	164796	India	200816	Myanmar	266933	Japan
122847	India	124112	India	164797	India	200817	Myanmar	266934	Japan
123187	India	125860	Afghanistan	166125	India	200819	Myanmar	266935	Japan
123188	India	125863	Afghanistan	166966	Turkey	204691	Turkey	266936	Japan
123493	India	125865	Afghanistan	167032	Turkey	207658	Japan	266937	Japan
123494	India	125866	Afghanistan	167044	Turkey	207660	Japan	266940	Japan
123495	India	125868	Afghanistan	167057	Turkey	212897	India	266941	Japan
123496	India	125869	Afghanistan	167058	Turkey	217523	Pakistan	266942	Japan
123498	India	125870	Afghanistan	167221	Turkey	217525	Pakistan	266943	Japan
123499	India	136169	Japan	167266	Turkey	217526	Pakistan	266945	Japan
123500	India	136173	China	179658	Turkey	217945	Pakistan	266947	Japan
123501	India	136186	China	179679	India	218070	Pakistan	266948	Japan
123502	India	149168	Russia	179680	India	218071	Pakistan	266949	Japan
123504	India	149169	Russia	179922	India	224688	Myanmar	267082	Russia
123505	India	154339	China	179923	India	224769	S. Korea	267862	Russia
123517	India	157070	China	180283	India	224786	Spain	268227	Iran
123680	India	157071	China	181749	Lebanon	234607	S. Africa	269367	Afghanistan
123681	India	157076	China	181750	Lebanon	247957	Finland	269368	Afghanistan
123682	India	157079	China	181875	Syria	249560	Thailand	269474	Pakistan
123687	India	157080	China	181941	Syria	251516	Iran	269477	China
269478	Pakistan	304746	El Salvador	357764	Yugoslavia	368538	Yugoslavia	401703	Spain
271757	Spain	304747	El Salvador	357765	Yugoslavia	368539	Yugoslavia	401708	Spain
274954	Senegal	306131	Iran	357767	Yugoslavia	368542	Yugoslavia	401709	Spain
275633	India	306135	Iran	357770	Yugoslavia	368543	Yugoslavia	403994	Colombia
275634	India	307610	Niger	357773	Yugoslavia	368544	Yugoslavia	408978	Myanmar
277282	India	313969	Russia	357774	Yugoslavia	369161	Lebanon	418768	Afghanistan
277283	India	313974	Russia	357775	Yugoslavia	374210	United States	419220	Poland
277284	India	314149	Russia	357786	Yugoslavia	374211	United States	420144	United States
280548	Israel	314150	Russia	357787	Yugoslavia	377586	France	420145	United States
282448	S. Africa	314170	Russia	357788	Yugoslavia	378170	Malaysia	420147	United States
288236	Egypt	314179	Russia	357791	Yugoslavia	381758	India	420148	United States
288330	India	314180	Russia	357797	Yugoslavia	381762	India	420149	United States
288331	Iran	314653	Russia	357798	Yugoslavia	385965	Kenya	420176	Japan
289876	Iran	315402	Russia	357800	Yugoslavia	385966	Kenya	420177	Japan
289885	Iran	315404	Russia	357805	Yugoslavia	390451	Ecuador	420178	Japan
292007	Israel	315409	Russia	357807	Yugoslavia	390452	Ecuador	420179	Japan
292009	Israel	315410	Russia	357814	Yugoslavia	401593	Spain	420180	Japan

North Central Regional Plant Introduction Station, Ames, Iowa, in 1991 (Table 1), and surveyed for isozyme variation using fifty-one enzyme systems (Table 2). Experimental germplasm included both melon subspecies *C. melo* L. subsp. *agrestis* (Naud.) and *C. melo* L. subsp. *melo* (i.e., cultivated groups *Cantalupensis* and *Inodorus*). Accessions possessing putative alleles of previously uncharacterized isozyme loci were used for inheritance and linkage studies (Table 3). Individuals possessing alternative alleles at putative isozyme loci were mated to produce heterozygous F_1 progeny. Selfing and backcrossing of these F_1 families produced segregating populations for linkage analysis.

SAMPLE PREPARATION AND ELECTROPHORESIS. Cotyledons of 15 individuals from each accession were harvested from 7-d-old seedlings. Samples were bulked for analysis such that ≈ 0.01 g of

cotyledonary tissue from each seedling was ground in 0.1 mL of a buffer solution containing $0.67 \text{ g}\cdot\text{L}^{-1}$ TRIS base and $7.02 \text{ g}\cdot\text{L}^{-1}$ TRIS-HCl at pH 7.1. Plant tissue was held at 5°C ($<2 \text{ h}$) before horizontal starch gel electrophoresis was performed according to Knerr and Staub (1992). Modified staining procedures of Allendorf et al. (1977), Brewer (1970), and Shaw and Prasad (1970) were used to visualize banding patterns of the 51 enzyme systems examined (Table 2).

Gels consisted of either 42 or 56 g of a 1:1:1 mixture of hydrolyzed potato starch from Sigma Co. (St. Louis), Connaught Laboratories (Willowdale, Ontario, Canada), and Starch Art (Smithville, Texas) dissolved in either 300 or 400 mL of buffer, respectively. Gel and electrode buffers described by Allendorf et al. (1977), Clayton and Tretiak (1972), Ridgway et al. (1970),

Table 1. Continued.

PI	Country	PI	Country	PI	Country	PI	Country	PI	Country
292310	Russia	321004	Taiwan	357816	Yugoslavia	401594	Spain	420181	Japan
292312	Russia	321005	Taiwan	357817	Yugoslavia	401599	Spain	426627	Pakistan
292313	Russia	345551	Russia	357821	Yugoslavia	401615	Spain	435086	China
293790	Russia	346788	United States	357822	Yugoslavia	401617	Spain	435087	China
293920	Israel	346789	United States	357823	Yugoslavia	401623	Spain	435285	Iraq
293921	Israel	346790	United States	357824	Yugoslavia	401655	Spain	435286	Iraq
293922	Turkey	355049	Iran	357827	Yugoslavia	401657	Spain	435287	Iraq
295341	Australia	355050	Iran	357829	Yugoslavia	401659	Spain	435289	Iraq
296118	Egypt	355051	Pakistan	358942	India	401670	Spain	435290	Iraq
296119	Egypt	357755	Yugoslavia	360713	Brazil	401672	Spain	435345	Mexico
296345	S. Africa	357756	Yugoslavia	368532	Yugoslavia	401674	Spain	435941	China
296386	Iran	357759	Yugoslavia	368533	Yugoslavia	401678	Spain	435992	China
300955	Afghanistan	357760	Yugoslavia	368534	Yugoslavia	401679	Spain	436531	Senegal
304745	El Salvador	357763	Yugoslavia	368537	Yugoslavia	401682	Spain	436532	Senegal
436533	Senegal	482394	Zimbabwe	503324	Turkey	537092	S. Korea		
436534	Senegal	482395	Zimbabwe	503325	Turkey				
438680	Mexico	482396	Zimbabwe	504522	India				
438685	Mexico	482397	Zimbabwe	504523	India				
438686	Mexico	482398	Zimbabwe	504524	India				
438687	Mexico	482399	Zimbabwe	504525	India				
439743	Iran	482400	Zimbabwe	504526	India				
439744	Iran	482401	Zimbabwe	504527	India				
439745	Greece	482402	Zimbabwe	504531	S. Korea				
439746	Cyprus	482403	Zimbabwe	504532	S. Korea				
439747	Cyprus	482407	Zimbabwe	505599	Zambia				
441987	Columbia	482408	Zimbabwe	505600	Zambia				
441988	Columbia	482410	Zimbabwe	505601	Zambia				
446928	Israel	482411	Zimbabwe	505602	Zambia				
446929	Israel	482413	Zimbabwe	505936	China				
446930	Israel	482414	Zimbabwe	507871	Hungary				
446931	Israel	482421	Zimbabwe	507873	Hungary				
470252	Indonesia	482422	Zimbabwe	508447	S. Korea				
476331	Russia	482424	Zimbabwe	508449	S. Korea				
476332	Russia	482427	Zimbabwe	508450	S. Korea				
476333	Russia	482429	Zimbabwe	508451	S. Korea				
476334	Russia	482430	Zimbabwe	508452	S. Korea				
476335	Russia	482431	Zimbabwe	512335	China				
476336	Russia	482433	Zimbabwe	525164	Egypt				
476337	Russia	482434	Zimbabwe	532822	China				
476338	Russia	489689	Mexico	532828	China				
476339	Russia	491200	Greece	532829	China				
476340	Russia	500362	Zambia	532831	China				
476341	Russia	502328	Russia	534536	Syria				
476342	Russia	502329	Russia	536479	Maldives				
482393	Zimbabwe	502330	Russia	536481	Maldives				

Table 2. Enzymes assayed using specific buffer systems that provided adequate resolution of isozyme loci observed in melon (*Cucumis melo* L.).

Enzyme	Abbreviation	EC designation ^z	Buffer system ^y	No. of putative loci observed ^x
Aconitase	AC	4.2.1.3	A	1
Acid phosphatase	ACP	3.1.3.2	C	2
Adenylate kinase	AK	2.7.4.3	S-4	1
Alanine aminotransferase	ALAT	2.6.1.2	R	--- ^w
Alcohol dehydrogenase	ADH	1.1.1.1	S-9	---
Aldolase	ALD	4.1.2.13	C	---
Alkaline phosphatase	AKP	3.1.3.1	C	---
Aspartate aminotransferase	AAT	2.6.1.1	R	---
Catalase	CAT	1.11.1.6	R	---
Diaphorase	DIA	1.6.4.1	C	---
Fumarase	FUM	4.2.1.2	M	---
Fructose diphosphatase	FDP	3.1.3.11	A	2
Fructokinase	FK	2.7.1.4	S-9	---
Hydroxybutyric dehydrogenase	HBDH	1.1.1.30	R	---
β-Galactosidase	β-GAL	3.2.1.23	C	---
Galactosaminidase	GAM	3.2.1.109	S-9	---
Glucose-6-phosphate dehydrogenase	G6PDH	1.1.1.49	C	---
Glucosephosphate isomerase	GPI	5.3.1.9	R	1
β-Glucosidase	β-GLU	3.2.1.21	C	---
Glutamate dehydrogenase	GDH	1.4.1.2	R	---
Glutathione reductase	GR	1.6.4.2	S-4	---
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	1.2.1.12	S-4	---
Glycerate dehydrogenase	G2DH	1.1.1.29	R	---
Glycerol-3-phosphate dehydrogenase	G3P	1.1.1.8	R	---
Glucokinase	GK	2.7.1.2	M	---
Hexosaminidase	HA	3.2.1.52	R	---
Isocitrate dehydrogenase	IDH	1.1.1.42	S-4	1
Lactate dehydrogenase	LDH	1.1.1.27	R	---
Leucine aminopeptidase	LAP	3.4.11.1	C	---
Mannitol dehydrogenase	MADH	1.1.1.67	S-4	---
Malate dehydrogenase	MDH	1.1.1.37	S-4	4
Malic enzyme	ME	1.1.1.40	C	---
α-Mannosidase	α-MAN	3.2.1.24	R	---
Manosephosphate isomerase	MPI	5.3.1.8	S-4	2
Methylumbelliferyl phosphatase	MUP	3.1.1.56	S-4	---
Octanol dehydrogenase	ODH	1.1.1.73	S-9	---
Peptidase with glycyl-leucine	PEPGL	3.4.13.11	A	1
Peptidase with leucyl-alanine	PEPLA	3.4.13.11	M	1
Peptidase with leucylglycyl-glycine	PEPLGG	3.4.13.11	R	---
Peptidase with phenylalanyl-proline	PEPPAP	3.4.13.11	S-4	1
Peroxidase	PER	1.11.1.7	A	---
6-Phosphogluconate dehydrogenase	PGD	1.1.1.43	S-4	1
Phosphoglucomutase	PGM	5.4.2.2	R	1
3-Phosphoglycerate kinase	PGK	2.7.1.10	C	---
Pyruvic kinase	PK	2.7.1.40	R	---
Phosphoglucokinase	PPGK	2.7.1.10	S-9	---
Sorbitol dehydrogenase	SDH	1.1.1.14	S-4	---
Shikimate dehydrogenase	SKDH	1.1.1.25	S-4	---
Superoxide dismutase	SOD	1.15.1.1	R	---
Triosephosphate isomerase	TPI	5.3.1.1	R	---
Xanthine dehydrogenase	XDH	1.1.1.204	M	---

^zEnzyme commission number.^yBuffers of Allendorf et al. (1977), Clayton and Tretiak (1972), Ridgway et al. (1970), Market and Faulhaber (1965) and Selander et al. (1971) designated as A, C, R, M and S, respectively where those indicated were the best buffers for band resolution.^xLoci designated using standard criteria and nomenclature (Richmond, 1972).^wEnzyme systems not resolved in this study.

Table 3. Sources of putative allozymic variation for inheritance and linkage studies in melon (*Cucumis melo* L.)².

Locus ³	PI	Source
<i>Ac</i> (2)	218071	Pakistan
	224769	Korea
<i>Acp-1</i> (2)	194057	China
	224786	Spain
<i>Acp-4</i> (1)	183256	Saudia Arabia
	224786	Spain
<i>Ak-4</i> (1)	169334	India
<i>Fdp-1</i> (2)	218071	Pakistan
	224688	Myanmar
<i>Fdp-2</i> (2)	204691	Turkey
	183256	Saudia Arabia
<i>Gpi</i> (1)	179680	India
<i>Idh</i> (1)	218070	Pakistan
	224688	Myanmar
<i>Mdh-2</i> (1) ³	224688	Myanmar
	224769	S. Korea
	218070	Pakistan
<i>Mdh-4</i> (2)	218070	Pakistan
	179923	India
<i>Mdh-5</i> (2) and -6(2)	179923	India
	180283	India
<i>Mpi-1</i> (1) and -2(1)	183257	Saudia Arabia
	204691	Turkey
<i>Pgd</i> (2)	218070	Pakistan
<i>Pgm</i> (2)	218070	Pakistan
	179923	India
<i>Pep-gl</i> (1)	218070	Pakistan
<i>Pep-la</i> (2)	183256	Saudia Arabia
<i>Pep-pap</i> (1)	183256	Saudia Arabia

²Sources are only given for the less common allele of a locus used based on previous studies by Knerr et al. (1989) and Knerr and Staub (1992).

³Enzyme loci designation where multiple loci of an enzyme is distinguished by hyphenated numerals and alleles are enclosed in parentheses. Allozymes that occurred in highest frequency were given the mobility designation 100. All other alleles produced protein products relative to allozyme 100 as follows: *Ac* (100, 104), *Acp-1* (100, 104), *Acp-4* (100, 96), *Ak-4* (100, 96), *Fdp-1* (100, 105), *Fdp-2* (100, 103), *Gpi* (100, 94), *Idh* (100, 95), *Mdh-2* (100, 96), *Mdh-4* (100, 104), *Mdh-5* (100, 105), *Mdh-6* (100, 103), *Mpi-1* (96, 100), *Mpi-2* (97, 100), *Pgd-1* (100, 108), *Pgm* (100, 104), *Pep-la* (100, 105), *Pep-gl* (100, 95), and *Pep-pap* (100, 96).

Market and Faulhauber (1965), and Selander et al. (1971) were used (Table 2). These are referred to respectively in the text as A (pH 7.1 gel, pH 7.0 electrode), C (pH 6.1 gel and electrode), M (pH 8.7 gel, 8.7 pH electrode), R (pH 8.5 gel, pH 8.1 electrode), and S-4 (pH 6.7 gel, pH 6.3 electrode).

ISOZYME LOCI. To determine the most appropriate buffer system for electrophoresis, cotyledons were taken from a random sampling of between 10 to 12 accessions in the USDA germplasm collection. Sample extracts and an array of buffers (A, C, M, R, and S-4) were then used to evaluate enzyme systems. Subsequently, a random set totalling 400 ($\approx 10\%$; Table 1) of the accessions in the USDA germplasm collection was evaluated for variation using 51 enzyme systems (Table 2).

Based on this initial survey, isozyme banding patterns observed in 13 enzyme systems (AC, ACP, AK, FDP, GPI, IDH, MDH, MPI, PEP-GL, PEP-LA, PEP-PAP, PGD, and PGM) were analyzed in 8 F₂ and 11 BC₁ families depending on observed polymorphisms. 'Top Mark' melon was used as a standard on each gel to provide cross-comparative estimates for allozyme mobilities. Genetic nomenclature follows Knerr and Staub (1992) modified from Richmond (1972). 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 the allele 100. For example, allele 2 at locus 1 of FDP that had a mobility 5 mm less than the most common allele (1) was assigned the designation *Fdp-1* (1) -95.

Individuals possessing alternate alleles for *Ac*, *Acp-1*, *Acp-4*, *Ak-4*, *Fdp-1*, *Fdp-2*, *Gpi*, *Idh*, *Mdh-2*, *Mdh-4*, *Mdh-5*, *Mdh-6*, *Mpi-1*, *Mpi-2*, *Pep-gl*, *Pep-la*, *Pep-pap*, *Pgd*, and *Pgm* were mated to produce F₁ individuals. Sources of putative allozyme variation and initial matings are listed in Tables 3 and 4. Heterozygous F₁ plants were self-pollinated to produce F₂ families and crossed to a recurrent parent (homozygous for one of the alternate alleles at the same locus) to provide BC₁ families. Results of BC₁ progeny analysis were compared to those obtained from F₂ family segregation patterns to determine the inheritance of allozymes.

LINKAGE ANALYSIS. Data were obtained for codominant isozyme

Table 4. Parental origin and associated isozyme loci of genetic stocks used in initial matings for genetic map construction of melon (*Cucumis melo* L.).

No.	Parent ²	Locus symbol ³	Mating partner(s) ⁴
1	169334	<i>Ak-4</i>	1
2	179680	<i>Acp</i> , <i>Gpi</i> , <i>Mdh-2</i> , <i>Pgd-1</i>	2, 3, 5
3	179923	<i>Fdp-1</i> , <i>Gpi</i> , <i>Mdh-4</i> , <i>Mdh-5</i> , <i>Mdh-6</i> , <i>Pgm</i> , <i>Pgd-1</i> , <i>Pgd-2</i>	3, 5
4	180283	<i>Mdh-5</i> , <i>Mdh-6</i>	4
5	183256	<i>Fdp-2</i> , <i>Gpi</i> , <i>Mdh-1</i> , <i>Mdh-2</i> , <i>Mpi-2</i> , <i>Pep-la</i> , <i>Pep-pap</i> , <i>Pgd-1</i> , <i>Pgd-2</i> , <i>Pgm</i>	2, 3
6	183257	<i>Acp</i> , <i>Fdp-4</i> , <i>Pep-pap</i> , <i>Pgd-1</i> , <i>Pgm</i> , <i>Mdh-2</i> , <i>Mdh-3</i> , <i>Mpi-1</i> , <i>Mpi-2</i>	7, 12
7	194057	<i>Acp</i> , <i>Fdp-4</i> , <i>Pep-pap</i> , <i>Pgd-1</i> , <i>Pgm</i> , <i>Mdh-2</i> , <i>Mdh-3</i> , <i>Mpi</i>	6, 8
8	204691	<i>Acp</i> , <i>Fdp-1</i> , <i>Fdp-2</i> , <i>Mpi-1</i> , <i>Mpi-2</i>	8, 7, 11
9	218070	<i>Ak</i> , <i>Idh</i> , <i>Mdh-1</i> , <i>Mdh-2</i> , <i>Mdh-4</i> , <i>Pep-gl</i> , <i>Pep-pap</i> , <i>Pgd</i> , <i>Pgm</i>	9
10	218071	<i>Acp</i> , <i>Fdp-1</i> , <i>Idh</i> , <i>Pgd-1</i>	11,
11	224688	<i>Ac</i> , <i>Acp</i> , <i>Fdp-1</i> , <i>Idh</i> , <i>Mdh-2</i> , <i>Mdh-3</i> , <i>Pgd-1</i>	8, 10, 11, 12
12	224769	<i>Ac</i> , <i>Fdp-1</i> , <i>Idh</i> , <i>Mdh-2</i> , <i>Mpi-1</i> , <i>Pep-gl</i> , <i>Pgd-1</i> , <i>Pgd-2</i>	11
13	224786	<i>Ac</i> , <i>Acp</i> , <i>Mdh-1</i> , <i>Mdh-2</i> , <i>Mdh-3</i> , <i>Pep-gl</i> , <i>Pep-la</i> , <i>Pep-pap</i> , <i>Pgm</i>	6

²Accessions (plant introductions = PI) received from U.S. North Central Plant Introduction Station, Ames, Iowa.

³Enzyme loci designation where multiple loci of an enzyme is distinguished by hyphenated numerals.

⁴No symbol in a mating partner indicates that alternate allele was present.

loci from F₂ and BC families and analyzed by chi-square analysis for conformity to expected 1:2:1 (df=2) and 1:1 (df=1) Mendelian single-factor segregation ratios. Homogeneity chi-squares were calculated for each segregating locus from pooled family (n) totals (df = n - 1). Data from putative loci which showed significant deviation from expected segregation ratios ($P < 0.001$) were excluded from linkage analysis.

Linkage and orders of markers were estimated with MapMaker version 2.0 for Macintosh computers (Lander et al., 1987). Marker loci were initially associated with two-point comparisons with threshold LOD 3.0 and $r = 0.30$. Loci were then placed in sequence via three-point analyses. Scoring errors were detected by checking for double crossovers. Final candidate orders were confirmed with multipoint analyses using the more stringent LOD of 4.0. A final map depicting relationships among isozyme loci was constructed using information from 19 individual linkage groups (maps) with common markers as anchor points.

Results and Discussion

ISOZYME VARIATION. Nineteen polymorphic isozyme banding regions (putative loci) were identified in thirteen enzyme systems (Table 2). Putative loci were identified at *Ac* (100, 104), *Acp-1* (100, 104), *Acp-4* (100, 96), *Ak-4* (100, 96), *Gpi* (100, 94), *Idh* (100, 95), *Mpi-1* (96, 100), *Mpi-2* (97, 100), *Pep-la* (100, 105), *Pep-gl* (100, 95), and *Pep-pap* (100, 96), *Pgd* (100, 108), and *Pgm* (100, 104). Polymorphisms in FDP are coded by two loci with two alleles [*Fdp-1* (100, 105), *Fdp-2* (100, 103)]. One enzyme, MDH, coded for four loci each with two alleles [*Mdh-2* (100, 96), *Mdh-4* (100, 104), *Mdh-5* (100, 105), *Mdh-6* (100, 103)].

SINGLE FACTOR SEGREGATION ANALYSIS. Segregation at nineteen previously undescribed isozyme loci in the F₂ and BC families agreed with the expected 1:2:1 and 1:1 segregation ratios ($P < 0.01$) (Tables 5 and 6). Although segregations for some loci (*Acp-4*, *Fdp-2*, *Mdh-4*, *Mdh-5*, *Pgd*, and *Pgm*) were distorted in some F₂ families, other F₂ and BC families segregated in predicted ratios indicating a monogenic inheritance for these loci.

Distorted segregations observed in some families could be due to progeny misclassification in difficult-to-interpret enzyme systems (Tables 5 and 6). For instance, interpretation of MDH zymograms (e.g., *Mdh-4* and *Mdh-5*) was made difficult by the complex banding patterns inherent with this system. More heterozygotes were identified in some F₂ (e.g., *Mdh-4*, family 4) and BC (e.g., *Mdh-4*, family 1) families than would have been expected by chance. Zymogram banding complexity at *Mdh-4* in cucumber also makes phenotype classification difficult (Knerr et al., 1995). Thus, it is essential that several segregating families be used in examining genetic variation in such enzyme systems.

Inviability, late germination or poor vigor of certain genotypes could also have led to distorted progeny segregation since only 15 of the ~20 seedlings planted were used for electrophoresis (99% confidence of detecting a heterozygote if it were present). Moreover, many of the original PIs used in crossing were exotic accessions (e.g., *C. melo* L. subsp. *agrestis*) having sex expression and flowering characteristics (e.g., andromonoecious) that were difficult to manipulate, and mineral fertilization regimes were difficult to optimize often. Such characteristics made the production of some F₂ and BC progeny impossible under our conditions. Thus, it is important to use germplasm with similar seed dormancy, flowering and complementary sex expression characteristics when developing genetic stocks for analysis of isozyme variation in melon.

LINKAGE ANALYSIS. Initially, a LOD of 3.0 and $r = 0.3$ were

chosen as thresholds for the identification of isozyme linkages in melon. This LOD corresponds to 95% confidence level against false detection of spurious linkages (Lander and Botstein, 1986). Loci were then placed in sequence via three-point analyses. Eighteen of twenty-eight pairwise comparisons, or 64% of the linkages between isozyme loci were placed on a map (i.e., final candidate orders confirmed by multipoint analyses) using an increased LOD stringency of 4.0 (LOD of 4.0 = 99.5% confidence).

This analysis resulted in the identification of a 98 cM map possessing two linkage groups (A and B). Linkage group A contains six loci and linkage group B contains five loci. Linkage order was as follows: **A** *Fdp-2*—4 cM—*Pgd*—29 cM—*Pgm*—8 cM—*Mpi-1*—1 cM—*Idh*—1 cM—*Ac*, and **B** *Pep-gl*—34 cM—*Mdh-2*—3 cM—*Mdh-4*—8 cM—*Mdh-5*—10 cM—*Mdh-6*. The average recombination value between loci was 8.9 cM. The greatest recombination fraction between any two isozyme loci was 34% (*Pep-al*—*Mdh-2*) and the smallest was 1% (*Mpi-1*—*Idh* and *Idh*—*Ac*). Eight loci (*Acp-1*, *Acp-4*, *Ak-4*, *Fdp-1*, *Gpi*, *Mpi-2*, *Pep-la* and *Pep-pap*) segregated independently from the other isozyme loci examined.

There are similarities in the order of isozyme loci in cucumber and melon. In cucumber, *Pgd-2* and *Fdp-2* are tightly linked (4 cM), and *Pgm*, *Mpi-1* and *Idh* are also closely linked (<4 cM) in another linkage group (Meglic and Staub, 1996). Similar to that observed in melon, *Mdh* loci (*Mdh-1*, *Mdh-2*, and *Mdh-3*) of cucumber were placed in the same linkage group. Such similarities in gene order might be expected given the relatively close genetic relationship of these *Cucumis* species.

In a melon map consisting of various marker types, 6-*Pgd-2* was loosely flanked by an RAPD and RFLP marker at ~22 cM and 44 cM, respectively (Baudracco-Arnas and Pitrat, 1996). This isozyme was placed on the same linkage group as *Fom-1*. Further genetic studies are required to determine whether 6-*Pgd-2* (Baudracco-Arnas and Pitrat, 1996) and *Pgd-1* (this study) are the same locus.

Tight linkages between biochemical genetic markers and traits of economic interest can be used to increase efficiency in plant improvement programs (Staub et al., 1997). Trait-isozyme linkage [e.g., *Pseudoperonospora cubensis* (Berk. and Curt.) Rostow causal agent of downy mildew and *Colletotrichum orbiculare* (Berk. & Mont.) Arx., causal agent of anthracnose] have been identified in cucumber (Meglic and Staub, 1996). Linkage has also been detected between RFLPs and economically important traits (e.g., downy mildew, *Cladosporium cucumerinum* Ellis and Arth., causal agent of scab and the female locus) in cucumber (Kennard et al., 1994). Likewise, relatively loose linkages (~5 to 20 cM) among genes for resistance to Fusarium wilt (*Fom-1* and *Fom-2*), melon necrotic spot virus (*nsv*), aphid infection (*Vat*), and molecular markers have been detected in melon (Baudracco-Arnas and Pitrat, 1996). It is likely that as more molecular polymorphisms are detected in melon, comprehensive genetic maps will be constructed. This will allow for a better understanding of the melon genome and an opportunity to test the utility of genetic markers for MAS in this species (Staub et al., 1996). However, marker saturation of the melon genome will be as difficult as that of cucumber. Although the level of polymorphism in melon (~10% to 15%) is higher than in commercial cucumber (~3% to 8%) (Staub et al., 1997), the estimated genomic length of melon (2276 to 3250 cM) is greater than that of cucumber (750 to 1000 cM) (Staub and Meglic, 1993). Depending on the method of calculation, the map of Baudracco-Arnas and Pitrat (1996) covers between 43% to 61% (~1390 cM) of the melon genome with an average marker interval of ~14 cM. Additional mapped markers will increase map saturation.

Table 5. F₂ single factor segregation for 19 enzyme coding loci in melon (*Cucumis melo* L.).

Enzyme locus ²	Observed family	Expected						χ^2	P
		AA	AB	BB	AA	AB	BB		
<i>Ac</i>	1	23	35	22	20	40	20	1.275	0.529
<i>Acp-1</i>	1	13	24	3	10	20	10	6.600	0.037
	2	10	31	7	12	24	12	4.458	0.108
	Pooled	23	55	10	22	44	22	11.058	
	Heterogeneity							1.717	0.190
<i>Acp-4</i>	1	18	38	22	19.5	39	19.5	0.461	0.794
	2	7	25	8	10	20	10	2.550	0.279
	3	15	25	8	12	24	12	2.125	0.346
	Pooled	40	88	38	41.5	83	41.5	5.136	
<i>Ak-4</i>								4.486	0.106
	1	9	22	17	12	24	12	3.000	0.223
	<i>Fdp-1</i>	17	25	19	15.2	30.5	15.2	2.188	0.335
	2	20	40	16	19	38	19	0.632	0.729
<i>Fdp-1</i>	Pooled	37	65	35	34.5	68.5	34.5	2.819	
	Heterogeneity							2.383	0.123
	<i>Fdp-2</i>	9	27	25	15.2	30.5	15.2	9.271	0.010
	2	6	25	9	10	20	10	2.950	0.229
<i>Fdp-2</i>	3	12	28	8	12	24	12	2.000	0.368
	Pooled	27	80	42	37.2	74.5	37.2	14.221	
	Heterogeneity							10.414	0.006
	<i>Gpi</i>	19	47	14	20	40	20	3.075	0.215
<i>Gpi</i>	2	22	43	15	20	40	20	1.675	0.433
	Pooled	41	90	29	40	80	40	4.750	
	Heterogeneity							0.450	0.502
<i>Idh</i>	1	23	37	20	20	40	20	0.675	0.741
	2	16	43	17	19	38	19	1.342	0.511
	Pooled	39	80	37	39	78	39	2.017	
	Heterogeneity							1.863	0.172
<i>Mdh-2</i>	1	16	41	23	20	40	20	1.275	0.529
	<i>Mdh-4</i>	19	40	21	20	40	20	0.100	0.951
	2	18	33	16	16.7	33.5	16.7	0.141	0.932
	3	18	22	21	15.2	30.5	15.2	5.146	0.078
<i>Mdh-4</i>	4	12	31	5	12	24	12	6.125	0.047
	Pooled	67	126	63	63.9	128	63.9	11.512	
	Heterogeneity							11.310	0.010
	<i>Mdh-5</i>	17	25	19	15.2	30.5	15.2	2.188	0.335
<i>Mdh-5</i>	2	18	19	3	10	20	10	11.350	0.003
	3	13	24	11	12	24	12	0.167	0.920
	Pooled	48	68	33	37.2	74.5	37.2	13.705	
	Heterogeneity							9.511	0.007
<i>Mdh-6</i>	1	19	31	17	16.5	33.5	16.5	0.523	0.770
	<i>Mpi-1</i>	25	39	16	20	40	20	2.075	0.354
	2	7	25	8	10	20	10	2.550	0.279
	3	17	42	17	19	38	19	0.842	0.656
<i>Mpi-1</i>	Pooled	49	106	41	49	98	48	5.467	
	Heterogeneity							3.508	0.173
	<i>Mpi-2</i>	25	37	14	19	38	19	3.237	0.198
	<i>Pgd</i>	14	27	13	13.5	27	13.5	0.037	0.982
<i>Pgd</i>	2	10	29	22	15.2	30.5	15.2	4.905	0.086
	3	18	40	18	19	38	19	0.211	0.900
	4	14	23	11	12	24	12	0.458	0.795
	Pooled	56	119	64	59.7	119.5	59.7	5.611	
<i>Pgm</i>								5.067	0.167
	1	24	27	10	15.2	30.5	15.2	7.297	0.026
	2	16	44	16	19	38	19	1.895	0.388
	Pooled	40	71	26	34.2	68.5	34.2	9.192	
<i>Pgm</i>								6.158	0.013
	<i>Pep-gl</i>	23	36	21	20	40	20	0.900	0.638
	2	17	29	15	15.2	30.5	15.2	0.299	0.861
	Pooled	40	65	36	35.2	70.5	35.2	1.102	
<i>Pep-gl</i>								0.083	0.774
	<i>Pep-la</i>	15	52	13	20	40	20	7.300	0.026
	<i>Pep-pap</i>	19	33	24	19	38	19	1.974	0.373

²Enzyme loci designation where multiple loci of an enzyme are distinguished by hyphenated numerals.

Table 6. Backcross single factor segregation for 15 enzyme coding loci in melon (*Cucumis melo* L.).

Enzyme locus	Family	Observed		Expected		χ^2	P
		AB	AA	AB	AA		
<i>Ac</i>	1	21	19	20	20	0.025	0.874
<i>Acp-1</i>	1	23	17	20	20	0.625	0.429
<i>Acp-4</i>	1	17	23	20	20	0.625	0.429
<i>Ak</i>	1	18	22	20	20	0.225	0.635
<i>Fdp-1</i>	1	24	16	20	20	1.225	0.268
	2	20	20	20	20	0.025	0.874
	3	18	22	20	20	0.225	0.635
	Pooled	62	58	60	60	1.475	
Heterogeneity						1.400	0.497
<i>Fdp-2</i>	1	19	21	20	20	0.025	0.874
	2	21	19	20	20	0.025	0.874
	Pooled	40	40	40	40	0.050	
Heterogeneity						0.038	0.847
<i>Gpi</i>	1	20	20	20	20	0.025	0.874
	2	20	20	20	20	0.025	0.874
	3	18	22	20	20	0.225	0.635
	4	19	21	20	20	0.025	0.874
	5	18	22	20	20	0.225	0.635
	Pooled	95	105	100	100	0.525	
Heterogeneity						0.120	0.998
<i>Idh</i>	1	22	18	20	20	0.225	0.635
	2	7	13	10	10	1.250	0.264
	3	18	22	20	20	0.225	0.635
	Pooled	47	53	50	50	1.700	
Heterogeneity						1.450	0.484
<i>Mdh-2</i>	1	21	19	20	20	0.025	0.874
<i>Mdh-4</i>	1	28	12	20	20	5.625	0.018
	2	20	20	20	20	0.025	0.874
	Pooled	48	32	40	40	5.650	
Heterogeneity						2.838	0.092
<i>Mdh-5</i>	1	23	17	20	20	0.625	0.429
<i>Mdh-6</i>	1	23	17	20	20	0.625	0.429
<i>Pgd</i>	1	18	22	20	20	0.225	0.635
	2	24	16	20	20	1.225	0.268
	3	21	19	20	20	0.025	0.874
	4	18	22	20	20	0.225	0.635
	5	17	23	20	20	0.625	0.429
	Pooled	98	102	100	100	2.325	
Heterogeneity						2.280	0.684
<i>Pgm</i>	1	16	24	20	20	1.225	0.268
	2	20	20	20	20	0.025	0.874
	3	19	21	20	20	0.025	0.874
	Pooled	55	65	60	60	1.275	
Heterogeneity						0.600	0.741
<i>Pep-la</i>	1	20	20	20	20	0.025	0.874

^aEnzyme loci designation where multiple loci of an enzyme are distinguished by hyphenated numerals.

tion. Toward this end, we have identified 19 isozyme loci and characterized their linkage relationships in melon.

Effective and efficient use of mapped markers will require the construction of saturated genetic maps (<5 cM between markers) (Staub et al., 1996). Map saturation can be increased by merging existing unsaturated maps to form consensus maps. However, this process is expensive and time consuming. Thus, coordination between melon mapping laboratories will become increasing important as more genetic information is acquired and consensus map building is contemplated.

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