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Genetic control and linkage relations of additional isozyme markers in chick-pea

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Summary. Allozyme polymorphisms of nine enzymes – aspartate aminotransferase (AAT), diaphorase (DIA), esterase (EST), formate dehydrogenase (FDH), β-galactosidase (GAL), β-glucosidase (GLU), malate dehydrogenase (MDH), malic enzyme (ME), and peroxidase (PRX) – were described in chick-pea (*Cicer* L.). Thirteen isozyme loci, *Aat-c, Dia-4, Est-2, Est-4, Est-10, Fdh, Gal-2, Gal-3, Gal-4, Glu-3, Mdh-2, Me-2,* and *Prx-2*, were genetically defined. Alleles of each of these isozyme loci expressed codominantly in heterozygotes and exhibited a codominant, single-locus segregation ratio in F_2. The loci *Est-2, Mdh-2*, and *Me-1* were expressed only in flower. Linkage relations were determined for these 13 and several previously defined isozyme loci. The following new genetic linkages were identified: *Pgm-p* (locus for plastid phosphoglucomutase) – *Est-10*; *Ald-p1* (one of the duplicate loci for plastid aldolase) – *Glu-3* – *Gal-2* – *Est-2,3; Gal-3* – *Aco-m* (locus for mitochondrial aconitase) – *Prx-2,3; Gpi-c* (locus for cytosolic glucosephosphate isomerase) – *Fdh*; and *Est-4* – *Me-1*. This study provides further confirmation on the existence of several conserved linkage groups among *Cicer, Pisum*, and *Lens*.

Key words: *Cicer* – Isozymes – Inheritance – Linkage – Conserved linkage groups

Introduction

Isozyme markers are widely used in gene mapping of crop plants. Nonepistatic expression of isozyme coding genes allows accumulation of several to many polymorphic isozyme loci in a single F_2 population, greatly increasing the efficiency of gene mapping. Furthermore, F_2 data on isozyme segregation provide a reliable estimate of recombination values, as the codominant expression of allozymes facilitates the identification of heterozygotes and both homozygotes. F_2 segregation of a single interspecific hybrid (also intraspecific hybrid in *Cicer*) has been used to determine simultaneously the linkage relationships of 14 isozyme loci in *Capsicum* (Tanksley 1984), 17 seed protein and isozyme loci in *Citrullus* (Navot and Zamir 1986), and over 20 isozyme loci in *Lycopersicon* (Tanksley and Rick 1980) and *Cicer* (Gaur and Slinkard 1990 and this report).

Chick-pea (*Cicer arietinum* L.), a self-pollinated diploid ($2n = 2x = 16$) of the tribe Cicereae and family Leguminosae, is an important food legume of the world, particularly on the Indian subcontinent. Genetic studies in chick-pea have focused mainly on determining inheritance of morphological traits. A few linkages between morphological trait loci have been identified (Bhat and Argikar 1951; Aziz et al. 1960; Bhapkar and Patil 1963; Deshmukh et al. 1972; Reddy and Chopde 1977a, b; Pawar and Patil 1979; Rao et al. 1980), but none of the loci was assigned to a specific chromosome.

We decided to use isozyme markers for identifying linkage groups of isozyme and morphological trait loci in chick-pea. In an earlier publication, we described inheritance for 16 isozyme loci and reported four linkage groups consisting of 13 of these isozyme loci (Gaur and Slinkard 1990). Subsequently, three morphological trait loci and two isozyme loci were assigned to the fifth linkage group of chick-pea (P. M. Gaur and A. E. Slinkard, communicated). This report describes inheritance for 13 additional isozyme loci. Two new linkage groups are reported and additional isozyme loci are assigned to existing linkage groups.

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Materials and methods

The *Cicer* lines used in this study included five accessions of *C. arietinum* – Macarena (A1), Mission (A2), ICC 5316 (A3), ICC 4957 (A4), and Radhe (A5); six accessions of *C. reticulatum* – ICCW 6 (R1), ICCW 8 (R2), ICCW 9 (R3), ICCW 9A (R4), PI 489777 (R5), and PI 489778 (R6); and one accession of *C. echinospermum* – PI 489776 (E1). The seeds of all PI accessions were obtained from the United States Department of Agriculture, Regional Plant Introduction Station, Pullman, WA. The seeds of all ICC and ICCW accessions and Radhe were obtained from the International Crop Research Institute for the Semi-Arid Tropics, Patancheru, India. The details of plant culture and crossing technique have been reported earlier (Gaur and Slinkard 1990).

Twenty enzymes were analyzed using starch gel electrophoresis. These enzymes were: acid phosphatase (E.C. 3.1.3.2; ACP), aconitase (E.C. 4.2.1.3; ACO), alcohol dehydrogenase (E.C. 1.1.1.1; ADH), aldolase (E.C. 4.1.2.13; ALD), amylase (E.C. 3.2.1.-; AMY), aspartate aminotransferase (E.C. 2.6.1.1; AAT), diaphorase (E.C. 1.6.99.-; DIA), endopeptidase (E.C. 3.4.-.-; ENP), esterase (E.C. 3.1.1.-; EST), formate dehydrogenase (E.C. 1.2.1.2; FDH), β-galactosidase (E.C. 3.2.1.23; GAL), glucosephosphate isomerase (E.C. 5.3.1.9; GPI), β-glucosidase (E.C. 3.2.1.21; GLU), leucine aminopeptidase (E.C. 3.4.11.1; LAP), malate dehydrogenase (E.C. 1.1.1.37; MDH), malic enzyme (E.C. 1.1.1.40; ME), peptidase (E.C. 3.4 .-.-; PEP), peroxidase (E.C. 1.11.1.7; PRX), phosphoglucomutase (E.C. 5.4.2.2; PGM), and 6-phosphogluconate dehydrogenase (E.C. 1.1.1.44; PGD). Four different electrophoretic buffer systems were used. System I (lithium-borate, pH 8.1/TRIS-citrate, pH 8.4), System II (citrate-histidine, pH 6.5), and electrophoretic conditions for these two systems were reported earlier (Gaur and Slinkard 1990). System III was TRIS-citrate, pH 7.0, as described by Meizel and Markert (1967), and System IV was citrate-morpholine, pH 6.1, as described by Clayton and Tretiak (1972). For System III and System IV gels, electrophoresis was performed at 150 V for 3–5 h. In all systems, 12% starch gels were used.

The appropriate source tissue, optimal gel buffer system, and the staining protocol for ACP, ACO, ADH, AMY, AAT, ENP, EST, GPI, LAP, PRX, PGM, PGD (Gaur and Slinkard 1990), ALD (P. M. Gaur and A. E. Slinkard, communicated), and PEP (P. M. Gaur and A. E. Slinkard, communicated) have been reported earlier. LAP and ADH were analyzed earlier in System I (Gaur and Slinkard 1990), but were later analyzed in System III, since it provided better separation of the allozymes of these enzymes. FDH and MDH were analyzed in System I, GLU in System II, DIA and ME in System III, GAL in System IV, and EST in both System I and System IV. GAL was analyzed from young leaves; FDH from either 24-h, water-soaked cotyledons or fresh flowers; GLU from young roots; and MDH and ME from fresh flowers. EST was analzyed from both fresh flowers and young leaves. The extraction buffer used for these enzymes was the same as reported earlier (Gaur and Slinkard 1990). The recipes for staining of GLU, MDH, and ME were identical to and the recipes for staining of DIA, FDH, and GAL were slightly different from those suggested by Wendel and Weeden (1989). For DIA, the amount of 2,6 dichlorophenol-indophenol was reduced four fold (from 2 mg to 0.5 mg). For FDH, the amount of formic acid was increased 20-fold (from 100 mg to 2 g). The buffer used for GAL was 0.1 *M* Na-acetate, pH 4.5. Both fluorescent and colorimetric methods of staining were used for EST. The recipe for fluorescent stain was as reported earlier (Gaur and Slinkard 1990). The solution for colorimetric stain of EST included 50 ml 0.1 *M* potassium phosphate (pH 6.0), 50 mg each of α- and β-naphthylacetate (jointly dissolved in 3 ml acetone) and 50 mg Fast Blue RR salt.

Isozymes and their coding loci were designated following the guidelines suggested by Weeden (1988 a). Segregation and linkage analysis were performed on F_2 single-plant data using the computer program LINKAGE-1 (Suiter et al. 1983).

Results

Allozyme polymorphisms

Twenty enzymes were electrophoretically analyzed in five accessions of *C. arietinum*, six accessions of *C. reticulatum*, and one accession of *C. echinospermum*. The three *Cicer* species together exhibited 31 polymorphic isozyme loci. All 31 isozyme loci were monomorphic in *C. arietinum*, whereas 28 isozyme loci were polymorphic within *C. reticulatum*. Three isozyme loci were monomorphic within each species, but polymorphic among species. The accessions of *C. reticulatum* differed from one another for a minimum of 5 (R3 versus R4) to a maximum of 21 isozyme loci (R5 versus R6). As the five accessions of *C. arietinum* included in this study had similar allozymes for all isozymes, interspecific F_2 segregation of *C. arietinum* with *C. reticulatum* and *C. echinospermum* and intraspecific F_2 segregation of *C. reticulatum* were used to determine inheritance for these isozyme loci.

Electrophoretic patterns and inheritance of isozymes

Aspartate aminotransferase. Four isozymes of AAT were observed in leaf extracts of chick-pea. The isozyme AAT-1 was located in the microbodies, AAT-2 in the mitochondria, AAT-3 in the plastids, and AAT-4 in the cytosol (Gaur and Slinkard 1990). AAT-1 was monomorphic. The inheritance of AAT-2 and AAT-3 allozymes was described earlier (Gaur and Slinkard 1990). Two allozymes were observed for AAT-4. The fast allozyme of AAT-4, AAT-4a was present in some plants of the *C. reticulatum* accession ICCW 8 (R2). In System I, the fast and the slow allozymes of AAT-3 (AAT-3a and AAT-3b) overlapped the fast and the slow allozymes of AAT-4 (AAT-4a and AAT-4b), respectively. In System II, AAT-4a and AAT-4b were slower than AAT-3a and AAT-3b, respectively, however, AAT-3b overlapped AAT-4a and the bands were diffuse. Like AAT-2 and AAT-3, AAT-4 was also dimeric. The allozymes of AAT-4 exhibited simple Mendelian segregation (Table 1).

Diaphorase. Four zones of bands were observed for diaphorase in the extracts of leaves or cotyledons. The staining for diaphorase was very inconsistent, and was greatly affected by the amount of 2,6 dichlorophenol indophenol in the staining solution. Polymorphism was observed for the least anodal zone. The isozyme in this zone was designated as DIA-4 and its coding locus a

Table 1. Goodness-of-fit tests for 1:2:1 F_2 segregation of isozyme phenotypes in chick-pea (pooled data)

Isozyme	Alleles	No. families	F_2 phenotype[a]			No. plants	Goodness-of-fit χ^2	Heterogeneity χ^2
			FF	FS	SS			
AAT-4	*ab*	2	23	50	37	110	4.47	1.43
DIA-4	*ab*	2	23	64	29	116	1.86	1.04
EST-2	*ab*	3	39	77	23	139	5.30	2.38
EST-4	*ab*	4	48	108	54	210	0.51	3.54
EST-10	*ab*	3	40	85	45	170	0.29	3.37
FDH	*ab*	4	35	85	42	162	1.00	7.23
GAL-2	*ab*	5	67	143	62	272	0.72	4.25
	bc	2	20	48	28	96	1.33	1.80
GAL-3	*ab*	7	86	176	89	351	0.05	11.02
GAL-4	*bc*	3	45	92	32	169	3.33	2.68
GLU-3	*ab*	5	63	129	62	254	0.07	7.94
MDH-2	*ac*	5	60	131	55	246	1.24	8.22
ME-1	*ab*	4	49	99	41	189	1.11	4.05
	ac	2	19	45	20	84	0.45	0.84
	bc	3	33	80	33	146	1.34	1.59
PRX-2	*ab*	3	47	90	32	169	3.38	5.84
	ab	1	5	34	12	51	7.59*	–

* Significant at $P=0.05$

[a] FF = homozygotes for allele coding fast allozyme, FS = heterozygote, SS = homozygotes for allele coding slow allozyme

Note: Degrees of freedom for goodness-of-fit $\chi^2=2$; degrees of freedom for heterogeneity χ^2 = (no. of families $\times 2$) -2

Dia-4. Two allozymes, DIA-4a and DIA-4b, were identified for DIA-4. The allozyme DIA-4b was present in *Cicer* line R2 only. Inheritance of DIA-4 allozymes was studied in one F_2 family of *C. arietinum* × *C. reticulatum* and one F_2 family within *C. reticulatum*. Both F_2 families provided a good fit to the expected 1:2:1 ratio (Table 1), indicating that the allozyme variants of DIA-4 were under the control of a single locus with two codominant alleles. The isozyme was functionally monomeric.

Esterase. The banding patterns of EST observed on System I gels using fluorescent stain were described earlier (Gaur and Slinkard 1990). Esterase revealed several additional isozymes when assayed in gel buffer System IV. Fluorogenic staining of System IV gels revealed seven anodal and one cathodal isozymes of EST in leaf extracts. Of these, three isozymes, two anodal and one cathodal, were polymorphic. An additional EST isozyme was observed in flower extracts, which was polymorphic and the most anodal EST isozyme on System IV gels. This flower-specific isozyme was also resolved on System I gels where it was the second anodal isozyme. Thus, the flower-specific isozyme was designated EST-2 (EST-1 was the most anodal isozyme on System I gels, monomorphic, and not seen on System IV gels) and the earlier designated EST-2 isozyme (Gaur and Slinkard 1990), now the third anodal isozyme on System I gels, was renamed EST-3. The third anodal isozyme on System IV gels was polymorphic and designated EST-4. The cathodal EST isozyme on System IV gels was also polymorphic and designated EST-10. EST-4 and EST-10 isozymes were not resolved on System I gels.

System IV gels were also stained by colorimetric staining. Only three isozymes, two anodal and one cathodal, were stained in this method. The isozymes stained were EST-2, EST-4, and EST-10 (Fig. 1). In other words, all polymorphic EST isozymes, except EST-3, were stained colorimetrically. Thus, this staining method was preferred. The EST-3 isozyme that was not resolved by colorimetric staining was studied on System I gels using fluorogenic staining (Fig. 2). F_2 segregation data for EST-3 (specific to fluorogenic staining) allozymes were described earlier (Gaur and Slinkard 1990). This report presents F_2 data on inheritance of EST-2 (flower specific), EST-4, and EST-10 (cathodal) allozymes in chick-pea. Allozymes of each of these isozymes segregated in a 1:2:1 ratio in F_2 and thus were under the control of a single locus (Table 1). All three isozymes were functionally monomeric.

Formate dehydrogenase. The enzyme FDH was assayed from extracts of water-soaked cotyledons or fresh flowers, as it was weakly expressed in leaf tissues. All *Cicer* lines exhibited a single band for FDH except R5, which exhibited a pair of bands. Three allozymes were identified for FDH. All *Cicer* lines expressed FDH-c (least anodal), except E1 and R5. FDH-b (second anodal) was expressed in E1, whereas both FDH-a (most anodal) and FDH-b were simultaneously expressed in R5. F_2 families of hybrids involving R5 as one of the parents segregated

in a ratio of 1 two-banded:2 three-banded:1 single-banded zymotype of FDH (Fig. 3, Table 1). It could not be established whether the pair of bands observed in R5 was coded by two tightly linked loci, or whether one isozyme was a posttranscriptional modification of the other.

It was not possible to establish the quaternary structure of FDH from hybrids or F_2 progeny involving R5 as one of the parents. This is because the two bands of R5 were inherited together in F_1 and all F_2 plants. Thus, the quaternary structure of FDH was determined from a *C. reticulatum* × *C. echinospermum* hybrid and the few resulting F_2 plants (population size not large enough to be included for segregation analysis). The three-banded zymotype of heterozygotes suggested that FDH was functionally dimeric.

β-Galactosidase. A single GAL isozyme was observed on System I gels in the anodal region, whereas four GAL isozymes, two anodal and two cathodal, were observed on System IV gels. The single isozyme on System I gels and the most anodal isozyme on System IV gels were monomorphic and most likely identical; thus, they were designated GAL-1. On System IV gels, an alkaline buffer in the staining solution stained only GAL-1, whereas an acidic buffer stained all four GAL isozymes, although bands for GAL-1 were not as sharp as observed when stained with alkaline buffer. The second anodal, less cathodal, and more cathodal isozymes on System IV gels were polymorphic and designated GAL-2, GAL-3, and GAL-4, respectively. Three allozymes were identified for GAL-2 and two allozymes each for GAL-3 and GAL-4. Allozymes of each of these isozymes showed monogenic inheritance (Table 1). All three isozymes were functionally monomeric.

β-Glucosidase. The enzyme GLU was expressed only in roots. Three anodal and one cathodal isozymes were observed in young root extracts. All four isozymes were polymorphic, but staining for the first two anodal isozymes was inconsistent and, therefore, these two isozymes were not analyzed further. The least anodal and the cathodal isozymes were designated GLU-3 and GLU-4, respectively. The banding pattern of both isozymes were inherited together in the parents and F_2 populations. It could not be established whether these two isozymes are coded by two tightly linked loci, or whether one isozyme is a posttranscriptional modification of the other. A good fit to the expected 1:2:1 ratio was obtained for GLU-3 (and GLU-4, since they were inherited together) in both F_2 families of *C. arietinum* × *C. reticulatum* and all three families of *C. reticulatum* × *C. reticulatum* (Table 1); this suggests monogenic control of both the GLU-3 and GLU-4 allozymes. Each isozyme is functionally monomeric.

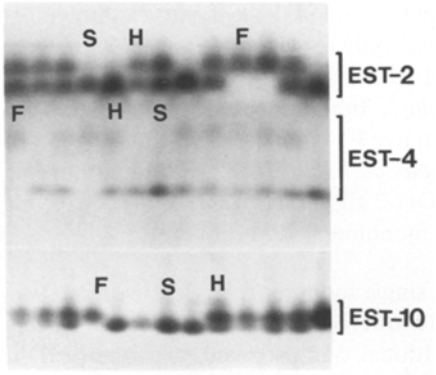

Fig. 1. A representative gel showing F_2 segregation of EST-2 (flower specific), EST-4, and EST-10 (cathodal) allozymes in *Cicer* (mixed sample of flower and leaf tissue). Anode is at the *top*. For each isozyme: *F* = homozygote of the alleles coding fast allozyme, *S* = homozygote of the alleles coding slow allozyme, and *H* = heterozygote

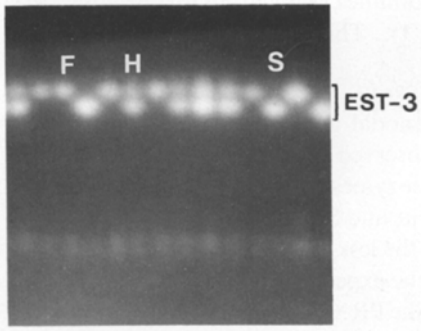

Fig. 2. A representative gel showing F_2 segregation of EST-3 (specific to fluorogenic staining) allozymes in *Cicer* (leaf tissue). Anode is at the *top*. *F* = homozygote of the alleles coding fast allozyme, *S* = homozygote of the alleles coding slow allozyme, and *H* = heterozygote

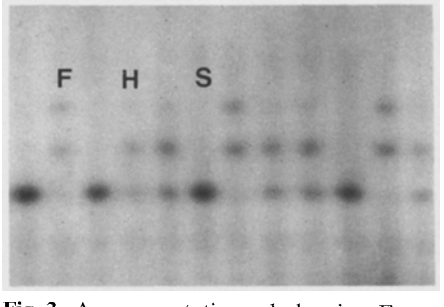

Fig. 3. A representative gel showing F_2 segregation of FDH allozymes in *Cicer* (24-h, water-soaked cotyledons). Anode is at the *top*. *F* = homozygote of the alleles coding fast allozyme, *S* = homozygote of the alleles coding slow allozyme and *H* = heterozygote. Note the comigration of the middle band with fast allozyme

Malate dehydrogenase. Four monomorphic bands were observed for MDH on System II gels when assayed from leaf tissues. On System I gels, MDH exhibited a single, wide band in leaf extracts and two bands in flower extracts. The additional band in the flower extracts was

cathodal to the single band in the leaf extracts. Polymorphism was observed only for the flower-specific isozyme of MDH, designated MDH-2. Three alleles were identified at locus *Mdh-2*. Inheritance was studied for '*ac*' alleles of *Mdh-2* in five F_2 families, which gave a good fit to the expected 1*aa*:2*ac*:1*cc* ratio, suggesting monogenic control of the MDH-2 allozymes (Table 1). This isozyme was functionally monomeric.

Malic enzyme. A single monomorphic ME isozyme was observed in leaf extracts of chick-pea. Anodal to this isozyme, an additional ME isozyme was observed in flower extracts. The additional isozyme expressed in flower extracts, designated ME-1, was polymorphic. The monomorphic isozyme (less anodal) was designated ME-2. Three allozymes were identified for ME-1. A normal Mendelian segregation was recorded for all four families segregating for '*ab*', both families segregating for 'ac', and all three families segregating for 'bc' allozymes of ME-1 (Table 1). This isozyme was functionally monomeric.

Peroxidase. Two anodal and one cathodal isozymes of peroxidase were observed in chick-pea leaf extracts. Inheritance of the allozymes of PRX-3 (cathodal) was described earlier (Gaur and Slinkard 1990). Two allozymes were identified for the less anodal isozyme PRX-2, which gave a good fit to the expected 1:2:1 ratio in three of the four F_2 families. Like PRX-3, PRX-2 was also monomeric.

Other enzymes. The banding patterns and F_2 segregation data of ACP, ACO, ADH, AMY, ENP, GPI, LAP, PGM, PGD (Gaur and Slinkard 1990), ALD (P. M. Gaur and A. E. Slinkard, communicated), and PEP (P. M. Gaur and A. E. Slinkard, communicated) isozymes have been described earlier.

Genetic linkages

Linkages between loci were detected by testing all possible pairs of loci for independent assortment in F_2 populations of chick-pea using the contingency χ^2 test. Recombination values were estimated only from those families that exhibited normal Mendelian segregation at both loci. Two new linkage groups were identified, and additional isozyme loci were assigned to three of five previously reported linkage groups (Fig. 4).

Linkage group I. Gaur and Slinkard (1990) identified a linkage group *Aat-p – Enp – Pgm-p* in *Cicer* and designated it as linkage group I. An additional isozyme locus *Est-10*, is now assigned to this linkage group. A tight linkage between *Pgm-p* and *Est-10* was detected (Table 2). No recombinant was observed between these two loci in any of the three families analyzed.

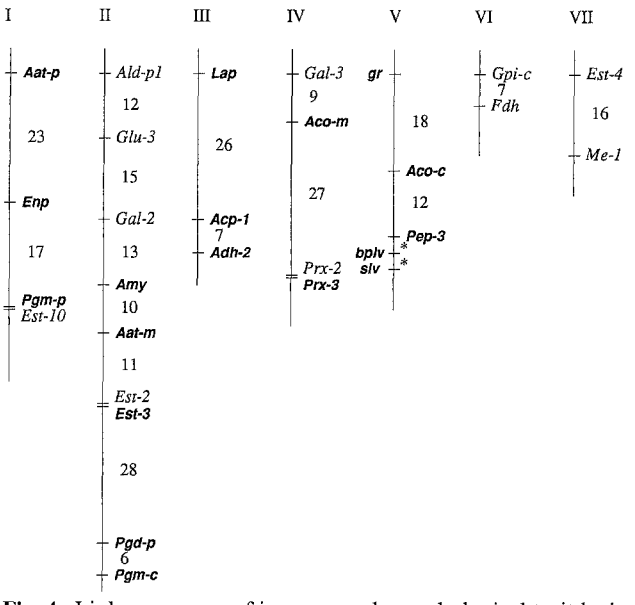

Fig. 4. Linkage groups of isozyme and morphological trait loci in *Cicer*. The linkage relations of loci shown in bold in linkage group I to IV (Gaur and Slinkard 1990) and in linkage group V (P. M. Gaur and A. E. Slinkard, communicated) have been reported earlier. (* An unbiased estimate of the map distance was not available)

Linkage group II. A linkage group *Amy – Aat-m – Est-3* (earlier designated *Est-2*) – *Pgd-p – Pgm-c* was identified in *Cicer* by Gaur and Slinkard (1990). This linkage group was designated linkage group II. Four additional isozyme loci are assigned to this linkage group (Fig. 4 and Table 2). *Ald-p1* was linked to *Glu-3* with a distance of 11.9 cMorgan and to *Gal-2* with a distance of 23.8 cMorgan. *Glu-3* was linked to *Gal-2* with a distance of 15.2 cMorgan, to *Amy* with a distance of 21.0 cMorgan, and to *Aat-m* with a distance of 27.4 cMorgan. *Gal-2* was linked to *Amy* with a distance of 13.3 cMorgan and to *Aat-m* with a distance of 22.4 cMorgan. The *Est-2* and *Est-3* loci were tightly linked and no recombinant was recovered between these two loci in any of the three families analyzed. These results and the results reported earlier (Gaur and Slinkard 1990) suggest the following gene order in this linkage group: *Ald-p1 – Glu-3 – Gal-2 Amy – Aat-m – Est-2,3 – Pgd-p – Pgm-c.*

Linkage group III. Gaur and Slinkard (1990) identified a linkage group *Lap – Acp-1 – Adh-2* and designated it linkage group III. No additional loci were assigned to this linkage group.

Linkage group IV. Linkage group IV of *Cicer* includes two isozyme loci, *Aco-m* and *Prx-3* (Gaur and Slinkard 1990). Two additional isozyme loci, *Gal-3* and *Prx-2*, are assigned to this linkage group (Fig. 4 and Table 2). A single F_2 family (R1 × R3) segregating for *Prx-2* and

Table 2. Contingency χ^2 tests for F_2 segregation data of pairs of loci exhibiting linkage in *Cicer*

Loci	Observed genotype frequencies[a]									χ^2	r+SE
Family	1/1	1/H	1/2	H/1	H/H	H/2	2/1	2/H	2/2		
Linkage group I											
Pgm-p/Est-10											
Pooled data	41	0	0	0	85	0	0	0	44	340.0***	0.0±ND
Heterogeneity										0.0	
Linkage group II											
Ald-p1/Glu-3											
(R5×R6)−2	8	2	0	0	14	2	1	3	10	41.4***	11.9±3.9
Ald-p1/Gal-2											
Pooled data	23	11	3	10	40	11	4	15	26	56.4***	23.8±3.0
Heterogeneity										5.1	
Glu-3/Gal-2											
Pooled data	18	5	3	5	39	3	0	9	18	77.3***	15.2±2.8
Heterogeneity										1.8	
Glu-3/Amy											
Pooled data	28	10	3	17	70	19	4	11	29	88.6***	21.0±2.4
Heterogeneity										5.2	
Glu-3/Aat-m											
Pooled data	18	5	3	7	27	13	2	13	12	34.2***	27.4±3.9
Heterogeneity										0.6	
Gal-2/Amy											
Pooled data	35	9	2	12	95	9	2	17	43	186.6***	13.3±1.7
Heterogeneity										13.5	
Gal-2/Aat-m											
Pooled data	27	20	4	10	75	17	3	21	32	83.8***	22.4±2.4
Heterogeneity										11.5	
Est-2/Est-3											
Pooled data	23	0	0	0	77	0	0	0	39	278.0***	0.0±ND
Heterogeneity										0.0	
Linkage group IV											
Gal-3/Aco-m											
Pooled data	28	8	1	7	74	5	1	7	41	187.1***	9.5±1.7
Heterogeneity										5.8	
Gal-3/Prx-2											
A3×R3	5	7	0	7	17	6	1	5	12	19.3***	25.5±4.8
Aco-m/Prx-2											
A3×R3	7	7	0	5	20	7	1	6	12	23.1***	23.4±4.4
Prx-2/Prx-3											
R1×R3	11	0	0	0	28	0	0	0	21	120.0***	0.0±ND
Linkage group VI											
Gpi-c/Fdh											
Pooled data	33	3	1	6	74	2	0	8	35	214.4***	6.7±1.4
Heterogeneity										4.0	
Linkage group VII											
Est-3/Me-2											
Pooled data	22	9	0	7	52	7	1	13	20	87.2***	15.9±2.5
Heterogeneity										0.3	

*** Significant at $P=0.001$

[a] 1 = homozygote for alleles of female parent; H = heterozygote; and 2 = homozygote for alleles of male parent

ND = not defined

Note: more detailed information is available upon request from the authors

Prx-3 showed no recombination between these two loci. PRX-2 and PRX-3 isozymes are coded by two different loci rather than one isozyme occurring as a posttranscriptional modification of the other, since different fingerprints were present for these isozymes in the parental lines, and many F_2 families were polymorphic for one isozyme and monomorphic for the other. The F_2 family A3 × R3 segregating for *Gal-3, Aco-m,* and *Prx-2* and the F_2 family (A3 × R6)-2 segregating for *Gal-3, Aco-m,* and *Prx-3* suggested a gene order of *Gal-3 – Aco-m – Prx-2,3* in this linkage group.

Linkage group V. Linkage group V consists of two isozyme loci, *Aco-c* and *Pep-3*, and three morphological trait loci, *gr* (green cotyledon color), *bplv* (bipinnate leaves), and *slv* (simple leaf) in the order *gr – Aco-c – Pep-3 – bplv – slv* (P. M. Gaur and A. E. Slinkard, communicated). No additional loci were assigned to this linkage group.

Linkage group VI. The loci *Gpi-c* and *Fdh* were tightly linked (Fig. 4 and Table 2) and assigned to linkage group VI. The distance between these two loci varied from 2.8 (A4 × R5) to 8.6 cMorgan [(R5 × R6) – 1]. The pooled data suggested a map distance of 6.7 cMorgan between *Gpi-c* and *Fdh.*

Linkage group VII. A linkage was detected between *Est-4* and the locus coding the flower-specific isozyme of ME, *Me-1* (Fig. 4 and Table 2). A map distance of 15.9 cMorgan was detected between these two loci. This linkage was assigned to linkage group VII.

Discussion

The genus *Cicer* was earlier classified in the tribe Vicieae Brown with the genera *Pisum, Lens, Vicia, Lathyrus,* and *Vavilovia* (Hutchinson 1964; Gunn 1969), but is now classified in its own tribe Cicereae Alef. (Kupicha 1977, 1981). Apparently, the tribe Cicereae and Vicieae are closely related. Only two genera of the tribe Vicieae, *Pisum* and *Lens*, have been subjected to extensive isozyme analysis. In *Pisum*, a recent review by Weeden (1988b) listed 50 isozyme loci for which monogenic inheritance has been demonstrated. In *Lens*, over 30 isozyme loci have been genetically defined (Zamir and Ladizinsky 1984; Tadmor et al. 1987; Havey and Muehlbauer 1989; Muehlbauer et al. 1989; Vaillancourt 1989). The genus *Cicer* has been subjected to less extensive isozyme analysis as compared to *Pisum* and *Lens*. Tuwafe et al. (1988) were the first to identify four isozyme loci in *Cicer*. Since then, monogenic inheritance has been demonstrated for an additional 12 isozymes (Gaur and Slinkard 1990). Two loci were identified for plastid-specific isozyme of ALD (P. M. Gaur and A. E. Slinkard, communicated). This report presents evidence on monogenic control of 14 additional allozyme polymorphisms in *Cicer.*

Several homologous isozyme loci and conserved linkages have been identified between *Pisum* and *Lens* (Weeden et al. 1988; Muehlbauer et al. 1989; Vaillancourt 1989). It was speculated that some homologous loci should exist between the tribe Vicieae and Cicereae (Weeden et al. 1988). Our earlier report (Gaur and Slinkard 1990) and this report suggest that several loci are indeed homologous between these two tribes. The number and subcellular distribution of the isozymes of GPI, PGM, PGD, and AAT in *Cicer* (Gaur and Slinkard 1990) are similar to those observed in *Pisum* (Weeden and Gottlieb 1980a, b) and *Lens* (Muehlbauer et al. 1989, Vaillancourt 1989). Moreover, the relative migration of these isozymes is also conserved, except for a slight change in the migration of mitochondrial and plastid isozymes of AAT. In *Cicer*, the mitochondrial AAT migrated faster than the plastid AAT (Gaur and Slinkard 1990), whereas the opposite was true for *Pisum* (Weeden and Gottlieb 1980a, b) and *Lens* (Muehlbauer et al. 1989; Vaillancourt 1989). However, a major disparity was found for the number of isozyme loci for plastid ALD. Two loci transcribe plastid ALD in *Cicer* (Kazan et al. 1989; P. M. Gaur and A. E. Slinkard, communicated), whereas only one locus transcribes plastid ALD in *Pisum* (Weeden and Gottlieb 1980b) and *Lens* (Muehlbauer et al. 1989).

Several other isozyme loci appear homologous among these genera. In *Pisum, Est-1* and *Est-2* were tightly linked and mapped between *Aat-m* and *Skdh-p* (Weeden and Marx 1987). In *Lens, Est-1* and *Est-3* were tightly linked and mapped near *Skdh-p* (Vaillancourt 1989). This study indicates that in *Cicer, Est-2* and *Est-3* are tightly linked and mapped near *Aat-m.* Thus, it appears that *Est-2* and *Est-3* of *Cicer* may be homologous to *Est-1* and *Est-2* of *Pisum* and to *Est-1* and *Est-3* of *Lens.* However, there is a difference in tissue-specific expression of these loci. In *Cicer* one of these tightly linked loci, *Est-2*, is expressed only in flowers, whereas in *Pisum* and *Lens* both loci are expressed in leaves. A comparison of leaf and flower EST has not been made in these legumes. It is also possible that one additional EST locus is present in this cluster.

Three GAL isozymes, one anodal and two cathodal, were observed in *Pisum* (Weeden 1985; Weeden and Marx 1987), whereas two GAL isozymes, one anodal and one cathodal, were observed in *Lens* (Muehlbauer et al. 1989). In both legumes, the anodal GAL isozyme had alkaline pH optima, whereas the cathodal isozymes had acidic pH optima. *Cicer* in this study had two anodal and two cathodal GAL isozymes. The most anodal isozyme, GAL-1, of *Cicer* may be homolgous to GAL-1 of *Pisum* and *Lens*, as it exhibited similar characteristics. The

Gal-2 of *Cicer* appears homologous to *Gal-2* of *Pisum*, as *Gal-2* is located close to *Amy* in both.

Weeden and Marx (1987) observed identical F_2 segregation for two isozymes of PRX, PRX-1 and PRX-2, in *Pisum*. From their available data, it could not be determined whether these two isozymes were coded by two tightly linked loci, or whether one isozyme was a post-transcriptional modification of the other. The tight linkage observed between *Prx-2* and *Prx-3* in *Cicer* suggests that PRX-1 and PRX-2 isozymes of *Pisum* are probably coded by two tightly linked loci. Thus, this linkage may also be conserved between *Pisum* and *Cicer*.

Several common linkage groups exist among *Cicer*, *Pisum*, and *Lens*. The linkage *Aat-p – Pgm-p* observed in *Cicer* (Gaur and Slinkard 1990) has also been reported in some species of *Lens* (Zamir and Ladizinsky 1984; Vaillancourt 1989). However, these two loci are located in different linkage groups in *Pisum* (Weeden and Marx 1987). Except for the location of the locus coding plastid ALD, the upper region of the linkage group II of *Cicer* (*Ald-p1 – Glu-3 – Gal-2 – Amy – Aat-m – Est-2,3*) is similar to the linkage group assigned on the upper region of chromosome 2 in *Pisum* (*Gal-2 – Amy – Ald-p – Aat-m Est-1,2 – Skdh-p*; Weeden and Marx 1987). Unfortunately, none of the loci of GLU has been mapped in *Pisum* and the locus of plastid SKDH, *Skdh-p*, has not been mapped in *Cicer*. A linkage group *Est-1,3 – Skdh-p – Pgd-p – Pgm-c* has been reported in *Lens* (Vaillancourt 1989). The linkage *Pgd-p – Pgm-c* has also been reported in *Pisum*, but it was present in an unassigned linkage group (Weeden and Marx 1987). These results suggest that the linkage group II is highly conserved among *Cicer*, *Pisum*, and *Lens*.

The linkage group *Lap – Acp-1 – Adh-2* observed in *Cicer* (Gaur and Slinkard 1990) appears homologous to the linkage group *Lap-2 – Acp-3 – Adh-1* reported in *Pisum* (Weeden and Marx 1987). However, the homology of loci of these linkage groups is uncertain since a second LAP locus is present in this linkage group in *Pisum*.

The linkage *Gpi-c – Fdh* in *Cicer* has also been reported in tepary bean, *Phaseolus acutifolius* (Garvin et al. 1989). Garvin et al. (1989) found that the locus *Fdh-1* was linked with *6Pgd-2*, which was linked with *Gpi-c1*. The linkage between *Fdh-1* and *Gpi-c1* was not analyzed, but linkage in common with *6Pgd-2* indicated that they are in same linkage group. In *Cicer*, locus *Gpi-c* is not linked to any of the loci of PGD. Thus, it appears that the parallel existence of the linkage *Gpi-c – Fdh* between *Cicer* and *Phaseolus* may be coincidental rather than conserved. There is no report on the genetics of FDH in *Pisum* or *Lens*.

This report and our earlier report (Gaur and Slinkard 1990) provide strong evidence that the genera (*Cicer*, *Pisum*, and *Lens* share many gene sequences in common. The existence of conserved gene sequences among these genera will facilitate prediction of location of a locus in one genus based on its location in the other genera. For example, the linkage of *Skdh-p* with two tightly linked loci of EST in *Pisum* and *Lens* suggests that *Skdh-p* of *Cicer* may also be located close to the two tightly linked EST loci in this genus. This approach will be particularly useful in attempts to tag commercially important genes in one genus based on their known linkage relationship with isozyme loci in another genus.

Within the last few years considerable progress has been made in identifying genetic linkages in *Cicer*. We have assigned 26 isozyme loci and 3 morphological trait loci to seven linkage groups (Fig. 4). Kazan and Muehlbauer (1989) have also identified several linkages between isozyme and morphological trait loci in *Cicer*. They reported that one of the loci of PGD was linked to the gene for round seed shape, one of the loci of ACP to the gene for brown seed coat, and one of the loci of ACO to the gene for angular seed type. Attempts to identify linkage between the loci controlling resistance to ascochyta blight (*Ascochyta rabiei*) and isozyme loci are in progress (Kusmenoglu et al. 1989).

Unfortunately, *C. arietinum*, the only cultivated species of the genus *Cicer*, exhibits only a few polymorphic isozyme loci. Oram et al. (1987) studied 20 accessions of *C. arientinum* representing 11 countries of origin for 20 enzymes (27 isozymes) and found only four polymorphic isozyme loci. They concluded that the cultivated chick-pea, as a species, is relatively poor in genetic variation at the isozyme loci. Tuwafe et al. (1988) studied 1,392 accessions of *C. arietinum* representing 25 countries of origin for six enzymes (total number of isozymes not reported) and found only four polymorphic isozymes. We analyzed 25 accessions of *C. arietinum* representing five countries of origin for 37 isozyme loci and found none of these isozyme loci to be polymorphic (P. M. Gaur and A. E. Slinkard, unpublished results). These results indicate that most isozyme loci are monomorphic in the cultivated chick-pea. However, these results are based on the survey of a limited number of accessions (our study and the study of Oram et al. 1987) or a limited number of enzymes (the study of Tuwafe et al. 1988). Further screening of germ-plasm with a large number of enzymes may identify more polymorphic isozyme loci in this species. Another approach to increasing isozyme polymorphisms in *C. arietinum* is by introgression of isozyme variants from *C. reticulatum*. These two species intercross readily and produce normal fertile progenies.

Until more polymorphic isozyme loci are identified/generated in the cultivated chick-pea, the application of isozyme markers in chick-pea appears limited to gene mapping, confirmation of interspecific hybrids, and monitoring interspecific gene introgression. The *C. reticulatum* accessions PI 489777 (R5) and PI 489778 (R6) differ from each other for at least 21 isozyme loci and

have proven useful tester stocks. These accessions will prove valuable in mapping genes for other morphological traits in *C. arietinum*.

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