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Isozyme polymorphism and phylogenetic interpretations in the genus *Cicer* L.

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Summary. Allozyme variation among 50 accessions representing the cultivated chickpea (*Cicer arietinum* L.) and eight wild annual *Cicer* species was scored and used to assess genetic diversity and phylogeny. Sixteen enzyme systems revealed 22 putative and scorable loci of which 21 showed polymorphism. Variation was prevalent between species ($D_{st}=0.510$) but not within species ($H_s=0.050$). No variation for isozyme loci was detected in the cultivated chickpea accessions. *Cicer reticulatum* had the highest proportion of polymorphic loci (0.59) while the loci *Adh*-2 and *Lap* were the most polymorphic over all the species accessions. The phylogeny of annual *Cicer* species, as determined by allozyme data, generally corroborated those based on other characters in previous studies. *Cicer arietinum*, *C. reticulatum* and *C. echinospermum* formed one cluster, while *C. pinnatifidum*, *C. bijugum* and *C. judaicum* formed another cluster. *Cicer chorassanicum* was grouped with *C. yamashitae*, whereas *C. cuneatum* formed an independent group and showed the largest genetic distance from *C. arietinum*.

Key words: *Cicer* – Domestication – Electrophoresis – Isozymes – Species relationships

Introduction

Enzyme electrophoresis is an effective tool for investigating phylogenetic relationships among related species and taxa. Analysis of isozyme variability in populations has provided useful information on the organization of the gene pool in a large number of plant species (Gottlieb 1981). Gel electrophoresis can be used to estimate divergence of particular genes in related species, and these data can be then applied to problems of systematics. Such studies have helped in the understanding of species relationships and phylogenies in *Glycine* (Verdc.) (Broué et al. 1977), *Vicia* (Yamamoto and Plitmann 1980; Yamamoto et al. 1982), *Lens* (Skibinski and Warren 1984; Pinkas et al. 1985; Hoffman et al. 1986), *Citrullus* (Navot and Zamir 1987), *Secale* (Vences et al. 1987), *Capsicum* (McLeod et al. 1983) and *Lycopersicum* (Rick and Fobes 1975).

Chickpea (*Cicer arietinum* L.), a self-pollinated diploid ($2n=2x=16$) of the tribe Ciceraea and family Leguminosae, is an important grain legume, particularly in the Indian subcontinent. There are eight wild annual *Cicer* species related to the cultivated chickpea, and their genetic relationships among themselves as well as with the cultivated species are poorly understood (van der Maesen 1987). Only a few electrophoretic studies have been reported on the genus *Cicer*, and these mostly deal with the cultivated species (Oram et al. 1987; Tuwafe et al. 1988; Gaur and Slinkard 1990a, b). Thus, in the present study isozyme variation in 16 different enzymes coded by 22 presumptive loci has been studied in all nine annual *Cicer* species. This information has been used to infer phylogenetic relationships among the annual *Cicer* species. These purported relationships are also compared with conclusions derived from other criteria such as interspecific crossability, chromosome pairing in interspecific hybrids, seed storage protein profile and morphological characters.

Materials and methods

Plants representing 25 accessions of the cultivated chickpea and 25 accessions of the eight wild annual *Cicer* species, *C. bijugum*

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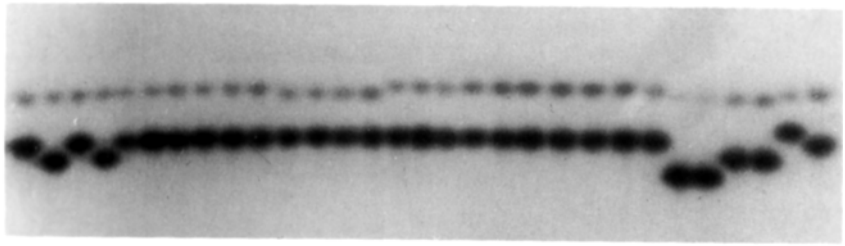

Fig. 1

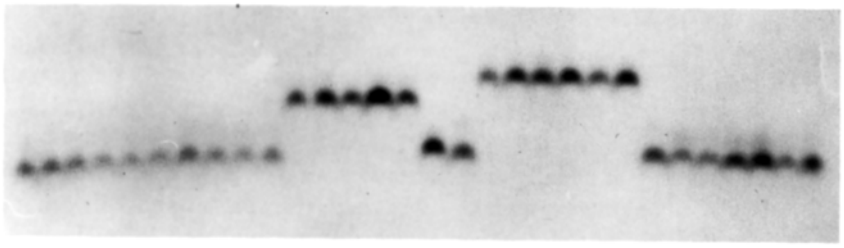

Fig. 2

Figs. 1–2. Electrophoregrams showing variation in isozyme banding pattern among annual *Cicer* species for (**1**) PGI (*Pgi-1* is the top faint zone and *Pgi-2* is the bottom dark zone), and (**2**) IDH. Anode is toward the *top*. Sample identification in various lanes is as follows (*L to R*): *1 C. arietinum* ILC 482, *2 C. reticulatum* PI 489777, *3 C. reticulatum* PI 489778, *4 C. reticulatum* ICCW 8, *5 C. reticulatum* ICCW 9, *6 C. reticulatum* ICCW 9a, *7 C. reticulatum* ICCW 6, *8 C. echinospermum* PI 489776, *9 C. echinospermum* ICCW 44, *10 C. arietinum* ICC 4934, *11 C. judaicum* ICCW 36, *12 C. judaicum* ICCW 35, *13 C. judaicum* ICCW 33, *14 C. judaicum* ICCW 34, *15 C. pinnatifidum* ICCW 40, *16 C. pinnatifidum* ICCW 37, *17 C. arietinum* cv 'Macarena' *18 C. bijugum* ICCW 7, *19 C. bijugum* ICCW 10, *20 C. bijugum* ICCW 41, *21 C. bijugum* ICCW 42, *22 C. bijugum* PI 458551, *23 C. bijugum* PI 458552, *24 C. arietinum* ICC 10301, *25 C. chorassanicum* PI 458553, *26 C. chorassanicum* ICCW 26, *27 C. yamashitae* ICCW 1, *28 C. yamashitae* ICCW 2, *29 C. cuneatum* ICCW 47, *30 C. arietinum* ILC 135

Rech. (2 accessions), *C. chrorassanicum* (Bge.) M. Pop (2 accessions), *C. cuneatum* Rich. (1 accession), *C. echinospermum* Dav. (2 accessions), *C. judaicum* Boiss. (4 accessions), *C. pinnatifidum* J. and S. (6 accessions), *C. reticulatum* Lad. (6 accessions) and *C. yamashitae* Kit. (2 accessions), were used for isozyme analysis. Accessions of the cultivated chickpea included cultivars and advanced breeding lines, while all of the accessions of the wild species were seed increases from material collected from natural habitats and had undergone several generations of selfing.

To assess intrapopulation variation in the wild *Cicer* species accessions, 10–12 plants from each accession were analysed electrophoretically. Five plants were sampled from each of the cultivated chickpea accessions. Starch gel electrophoresis, was used to examine the following 16 enzyme systems: aconitase (ACO, EC 4.2.1.3), alcohol dehydrogenase (ADH, EC 1.1.1.1), amylase (AMY, EC 3.2.1.1), aspartate aminotransferase (AAT, EC 2.6.1.1), catalase (CAT, EC 1.11.1.6), diaphorase (DIA, EC 1.6.4.3), endopeptidase (ENP, EC 3.4._._), β-galactosidase (GAL, EC 3.2.1.23), isocitrate dehydrogenase (IDH, EC 1.1.1.42), leucine amino peptidase (LAP, EC 3.4.11.1), malic enzyme (ME, EC 1.1.1.40), methyl umbelliferyl esterase (Mu-EST, EC 3.1.1.2), 6-phosphogluconate dehydrogenase (6-PGD, EC 1.1.1.44), phosphoglucose isomerase (PGI, EC 5.3.1.9), phosphoglucomutase (PGM, EC 5.4.2.2) and peroxidase (PRX, EC 1.11.1.7). Enzyme extraction, electrophoresis and enzyme assays were carried out according to the procedures of Gaur and Slinkard (1990a, b).

Nomenclature of the various isozymes and their alleles was followed according to Kazan et al. (1991). The allozymic nature of the variants at many of these loci has been previously verified by Gaur and Slinkard (1990a, b). Pending further analysis of the remaining isozyme loci, the electrophoretic variants within a region were considered putative allozymes.

On the assumption, that the populations in each of the species represented a random sample of the genetic variability in the taxon allele frequencies were calculated for various allozymes. Genetic identities (I) and genetic distances (D) were computed from allele frequencies for all possible pair-wise comparisons of the species following Nei (1975). Nei's genetic diversity statistics were also used to partition the total genetic diversity (H_t) into within (H_s) and between (D_{st}) species components and also to compute the coefficient of gene differentiation ($G_{st} = D_{st}/H_t$). Genetic distances were used to construct a dendrogram, using the UPGMA method of Sneath and Sokal (1973). The proportion of polymorphic loci per taxon and the average number of alleles per locus per taxon were also calculated.

Results and discussion

The 16 enzyme systems revealed 22 scorable isozyme loci, presumed products of individual coding loci. These were *Aat*, *Aco-1*, *Aco-2*, *Adh-1*, *Adh-2*, *Amy*, *Cat*, *Dia-1*, *Enp*, *Gal*, *Idh*, *Lap*, *Me*, *Mu-est*, *6-Pgd-1*, *6-Pgd-2*, *Pgi-1*, *Pgi-2*, *Pgm-1*, *Pgm-2*, *Prx-1* (anodal), *Prx-2* (cathodal). All scored loci, except *Prx-2* (cathodal) migrated anodally. Out of the 22 loci identified, 21 exhibited polymorphism, while the locus *Cat* was monomorphic. In total, 377 individuals were assayed in nine annual *Cicer* species, and all were homozygous for all isozymes except for 1 plant each of *C. reticulatum* accessions ICCW 6 and ICCW 9, which were heterozygous (1 locus each). Allozyme frequencies for the 22 isozyme loci in the nine annual *Cicer* species are presented in Table 1. Representative zymograms showing allozyme variation for the isozymes of PGI and IDH can be seen in Figs. 1 and 2, respectively.

While variation was prevalent between species ($D_{st}=0.510$), only a low level of variation was found within species ($H_s=0.050$). The coefficient of gene differentiation (G_{st}) was 0.911. The highest proportion of polymorphic loci (Table 2) was found in *C. reticulatum* (0.59), followed by *C. pinnatifidum* (0.32), while *C. bijugum* and *C. cuneatum* possessed a low level of polymorphism (0.05). The remaining annual *Cicer* species did not show any isozyme polymorphism (Table 2). Thus, *C. reticulatum* had the highest number of alleles per locus (1.68 ± 0.65), followed by *C. pinnatifidum* (1.32 ± 0.48) and *C. bijugum* and *C. cuneatum* (1.05 ± 0.21). The other species had only 1 allele per locus (Table 2).

Within *C. reticulatum*, the accessions PI 489777, PI 489778 and ICCW 9 were monomorphic (although fixed for different alleles), while the accessions ICCW 6, ICCW 8 and ICCW 9a were polymorphic at 4, 5 and 7 isozyme loci, respectively (data not presented). The two accessions of *C. pinnatifidum* were fixed for different alleles at the 7 polymorphic loci. In all cases, *C. pinnatifidum* accession ICCW 40 was fixed for the faster moving allele, while accession ICCW 37 was fixed for the slower moving allele at any given polymorphic locus. The isozyme loci *Mu-est* and *Adh-2* were polymorphic, respectively, in *C. cuneatum* and *C. bijugum*. *Adh-2* and *Lap* were the most polymorphic isozyme loci, while the locus *Gal* was the least polymorphic; *Cat* was monomorphic.

Cicer arietinum and *C. reticulatum* shared alleles for all isozyme loci except *Aco-2*, *6-Pgd-1* and *6-Pgd-2* (Table 1). The allozymes *Pgm-1b, c* and *Pgm-2b, c* were equally frequent in these two species, while the allozymes *Pgm-1c* and *Pgm-2b* were fixed in *C. arietinum*. *Cicer echinospermum* and *C. arietinum* were fixed for the same alleles at 14 of the 22 isozyme loci studied. *Cicer pinnatifidum* shared alleles with *C. arietinum* at 9 isozyme loci. The remaining *Cicer* species, *C. judaicum*, *C. bijugum*, *C. chorassanicum*, *C. yamashitae* and *C. cuneatum*, were fixed for 8, 10, 7, 8 and 5 alleles, respectively, that were common to *C. arietinum*.

Certain species apparently did not express activity for some isozymes. These were *Aco-1* and *Adh-2* in *C. cuneatum*, *Enp* in *C. chorassanicum* and *Lap* in *C. bijugum*. Also, individuals from *C. bijugum* accession ICCW 7 apparently did not express activity for *Adh-2* (data not presented). Comigration with other bands of ACO and ADH is possible, although the other bands in these individuals did not appear broader or of greater intensity. Even variations in the technique were unable to resolve the missing bands. Pending further investigations, these individuals were scored as "null" for the corresponding loci.

Based upon known and presumed genetic relationships of the banding patterns, Nei's (1975) genetic distances (D) for all pairwise combinations of the nine annual *Cicer* species were calculated and presented in Table 3. Small genetic distances were observed between *C. arietinum* and *C. reticulatum* ($D=0.242$) and between *C. reticulatum* and *C. echinospermum* ($D=0.267$). The largest genetic distance was observed between *C. judaicum* and *C. cuneatum* ($D=2.389$). In general, *C. cuneatum* showed large genetic distances with all the other eight annual *Cicer* species. The results show that *C. arietinum* is most closely related to *C. reticulatum*, followed by *C. echinospermum*, *C. bijugum*, *C. pinnatifidum*, *C. judaicum*, *C. chorassanicum*, *C. yamashitae* and *C. cuneatum*. These and other genetic distances are more easily visualized by examining the dendrogram (Fig. 3) derived from the cluster analysis (UPGMA) of the genetic distances.

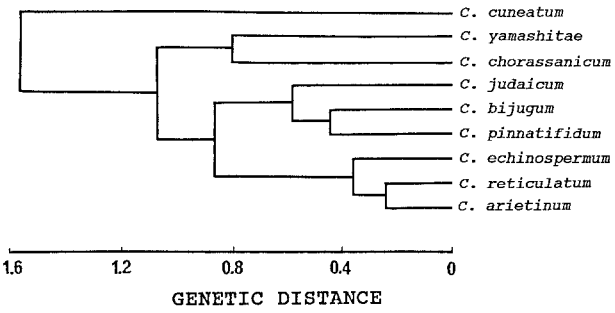

Fig. 3. Dendrogram showing relationships among the annual *Cicer* species, based on Nei's genetic distances from isozyme data

The most striking feature of the data is the limited variation generally found within each species (Table 2). Only four species, *C. reticulatum*, *C. pinnatifidum*, *C. bijugum* and *C. cuneatum*, showed some within species isozyme polymorphism. The polymorphism parameters of these nine species were compared to the values obtained from 28 naturally self-pollinating taxa (selfers) previously compiled by Gottlieb (1981). In *C. reticulatum* and *C. pinnatifidum*, the proportion of polymorphic loci was much greater than the average value for selfers (0.183). In comparison, it was much lower in *C. bijugum* and *C. cuneatum*, and non-existent in the remaining annual *Cicer* species. The average number of alleles per polymorphic locus in the four *Cicer* species that were polymorphic (2.04 ± 0.08) was lower than the average value calculated for other selfers (2.26). While polymorphism was random among accessions of *C. reticulatum*, it followed a specific pattern in *C. pinnatifidum*. Thus, in *C. pinnatifidum*, plants of the accessions ICCW 40 and ICCW 37 were invariably fixed for the fast and slow moving allele, respectively, at 7 polymorphic loci: *Aco-1*, *Adh-2*, *Amy*, *Idh* (Fig. 2), *Lap*, *Me* and *Prx-1* (anodal). No satisfactory explanation is yet available for the relatively high rate of polymorphism in *C. reticulatum* and *C. pinnatifidum* compared to the other annual *Cicer* species. There may be some association between polymorphism and geographical distribution or ecological factors such as altitude, bedrock or rainfall. However, it will be diffi-

Table 1. Allozyme frequencies in the nine annual *Cicer* species

Species[a]		ARI	RET	ECH	JUD	PIN	BIJ	CHO	YAM	CUN
Number of accessions		25	6	2	4	2	6	2	2	1
Number of plants		125	60	20	40	20	60	20	20	12
Locus	Allele[b]									
Aat	*a*	–	–	–	–	–	–	–	1.00	–
	b	–	–	–	–	–	–	1.00	–	–
	c	–	0.21	1.00	1.00	1.00	1.00	–	–	–
	d	1.00	0.79	–	–	–	–	–	–	1.00
Aco-1	*a*	–	0.17	–	–	–	–	–	1.00	–
	b	1.00	0.43	1.00	–	–	–	1.00	–	–
	c	–	0.40	–	–	0.50	1.00	–	–	–
	d	–	–	–	1.00	0.50	–	–	–	–
	n	–	–	–	–	–	–	–	–	1.00
Aco-2	*a*	–	0.50	–	–	1.00	1.00	–	–	–
	b	–	–	–	–	–	–	1.00	1.00	–
	c	–	–	–	1.00	–	–	–	–	–
	d	–	0.50	1.00	–	–	–	–	–	1.00
	e	1.00	–	–	–	–	–	–	–	–
Adh-1	*a*	–	–	1.00	–	–	–	–	–	1.00
	b	1.00	1.00	–	–	–	–	–	–	–
	c	–	–	–	–	–	–	–	1.00	–
	d	–	–	–	1.00	1.00	1.00	1.00	–	–
Adh-2	*a*	–	0.33	–	–	–	–	–	–	–
	b	–	–	–	–	0.50	–	–	–	–
	c	–	–	–	–	–	–	1.00	–	–
	d	1.00	0.50	1.00	1.00	0.50	0.83	–	1.00	–
	e	–	0.17	–	–	–	–	–	–	–
	n	–	–	–	–	–	0.17	–	–	1.00
Amy	*a*	–	–	–	–	0.50	–	–	–	–
	b	–	0.44	1.00	–	0.50	–	–	1.00	–
	c	1.00	0.56	–	1.00	–	1.00	1.00	–	–
	d	–	–	–	–	–	–	–	–	1.00
Cat	*a*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dia-1	*a*	1.00	1.00	1.00	–	1.00	1.00	–	–	–
	b	–	–	–	1.00	–	–	–	–	–
	c	–	–	–	–	–	–	–	–	1.00
	d	–	–	–	–	–	–	1.00	1.00	–
Enp	*a*	–	–	–	–	–	1.00	–	–	1.00
	b	1.00	1.00	–	1.00	1.00	–	–	1.00	–
	c	–	–	1.00	–	–	–	–	–	–
	n	–	–	–	–	–	–	1.00	–	–
Gal	*a*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–	–
	b	–	–	–	–	–	–	–	1.00	1.00
Idh	*a*	–	–	–	–	–	1.00	–	–	–
	b	–	–	–	1.00	0.50	–	–	–	–
	c	1.00	1.00	1.00	–	0.50	–	1.00	1.00	1.00
Lap	*a*	–	0.17	–	–	–	–	1.00	–	–
	b	–	–	–	–	0.50	–	–	1.00	1.00
	c	–	–	–	–	0.50	–	–	–	–
	d	1.00	0.83	1.00	–	–	–	–	–	–
	e	–	–	–	1.00	–	–	–	–	–
	n	–	–	–	–	–	1.00	–	–	–
Me	*a*	–	–	–	–	0.50	–	–	–	–
	b	1.00	1.00	1.00	1.00	–	1.00	1.00	1.00	1.00
	c	–	–	–	–	0.50	–	–	–	–
Mu-est	*a*	–	–	–	–	–	–	–	–	0.75
	b	1.00	0.53	1.00	–	–	–	–	1.00	–
	c	–	0.47	–	–	–	–	–	–	0.25
	d	–	–	–	1.00	1.00	1.00	1.00	–	–

Table 1. (Continued)

Species[a]		ARI	RET	ECH	JUD	PIN	BIJ	CHO	YAM	CUN
Number of accessions		25	6	2	4	2	6	2	2	1
Number of plants		125	60	20	40	20	60	20	20	12
Locus	Allele[b]									
6-Pgd-1	*a*	–	0.83	1.00	1.00	1.00	–	1.00	1.00	–
	b	–	–	–	–	–	–	–	–	1.00
	c	1.00	–	–	–	–	1.00	–	–	–
	d	–	0.17	–	–	–	–	–	–	–
6-Pgd-2	*a*	–	–	–	–	–	–	1.00	1.00	1.00
	b	–	1.00	1.00	–	–	–	–	–	–
	c	1.00	–	–	–	–	–	–	–	–
	d	–	–	–	1.00	1.00	1.00	–	–	–
Pgi-1	*a*	1.00	1.00	1.00	–	1.00	1.00	–	–	1.00
	b	–	–	–	1.00	–	–	1.00	1.00	–
Pgi-2	*a*	–	–	–	–	–	–	–	–	1.00
	b	1.00	0.70	1.00	1.00	1.00	1.00	–	–	–
	c	–	0.30	–	–	–	–	–	1.00	–
	d	–	–	–	–	–	–	1.00	–	–
Pgm-1	*a*	–	–	–	1.00	1.00	1.00	1.00	–	–
	b	–	0.50	1.00	–	–	–	–	1.00	1.00
	c	1.00	0.50	–	–	–	–	–	–	–
Pgm-2	*a*	–	–	–	1.00	–	1.00	1.00	1.00	–
	b	1.00	0.50	1.00	–	1.00	–	–	–	–
	c	–	0.50	–	–	–	–	–	–	1.00
Prx-1	*a*	–	–	–	–	–	–	–	–	1.00
anodal	*b*	–	–	–	–	0.50	–	–	–	–
	c	–	–	–	1.00	0.50	–	–	–	–
	d	–	0.17	–	–	–	–	–	–	–
	e	1.00	0.83	1.00	–	–	1.00	1.00	1.00	–
Prx-2	*a*	–	–	–	–	–	–	1.00	–	–
cathodal	*b*	1.00	0.67	1.00	1.00	–	–	–	1.00	–
	c	–	0.33	–	–	1.00	1.00	–	–	–
	d	–	–	–	–	–	–	–	–	1.00

[a] ARI, *C. arietinum*; RET, *C. reticulatum*; ECH, *C. echinospermum*; JUD, *C. judaicum*; PIN, *C. pinnatifidum*; BIJ, *C. bijugum*; CHO, *C. chorassanicum*; YAM, *C. yamashitae*; CUN, *C. cuneatum*

[b] Relative mobility of allele: $a>b>c>d>e$; the null allele is represented by *n*

Table 2. Proportion of polymorphic loci and average number of alleles per locus in annual *Cicer* species

Species	Proportion of polymorphic loci[a]	Average number of alleles per locus
C. arietinum	0.00	1.00±0.00
C. reticulatum	0.59	1.68±0.65
C. echinospermum	0.00	1.00±0.00
C. judaicum	0.00	1.00±0.00
C. pinnatifidum	0.32	1.32±0.48
C. bijugum	0.05	1.05±0.21
C. chorassanicum	0.00	1.00±0.00
C. yamashitae	0.00	1.00±0.00
C. cuneatum	0.05	1.05±0.21

[a] A locus is polymorphic if the frequency of the most common allele is equal to or less than 0.99 (Nei 1975)

cult to show such an association, since only a few, accessions of wild *Cicer* species are present in the worlds germ plasm. Another possible explanation for the observed differences in polymorphism could be rare spontaneous introgression among *C. reticulatum*, *C. arietinum* and *C. echinospermum* on one hand and between *C. pinnatifidum* and *C. judaicum* on the other hand. However, in this case it would be difficult to comprehend the unidirectional flow of genes.

Among 377 plants, representing 50 accessions of nine annual *Cicer* species, only 2 plants of *C. reticulatum* were heterozygous (1 each of accession ICCW 6 and ICCW 9a for the locus *Amy* and *Mu-est*, respectively). This translates to a mean observed heterozygosity of 0.033 in *C. reticulatum* or 0.005 in the annual *Cicer* species. A low level of heterozygosity was, however, expected since

Table 3. Nei's genetic distances, based on presumed genetic relationships derived from the observed isozyme banding patterns, between all possible pairs of the nine annual *Cicer* species

Species[a]	ARI	RET	ECH	JUD	PIN	BIJ	CHO	YAM
RET	0.24 (0.09)[b]							
ECH	0.45 (0.17)	0.27 (0.10)						
JUD	1.01 (0.29)	0.93 (0.26)	1.01 (0.29)					
PIN	0.93 (0.27)	0.63 (0.18)	0.75 (0.22)	0.52 (0.17)				
BIJ	0.80 (0.24)	0.74 (0.21)	0.91 (0.27)	0.61 (0.20)	0.44 (0.16)			
CHO	1.15 (0.32)	1.02 (0.28)	1.15 (0.32)	0.79 (0.24)	1.13 (0.32)	0.89 (0.26)		
YAM	1.01 (0.29)	0.77 (0.21)	0.79 (0.24)	1.01 (0.29)	1.40 (0.35)	1.51 (0.41)	0.79 (0.24)	
CUN	1.47 (0.40)	1.07 (0.29)	1.14 (0.32)	2.39 (0.69)	1.90 (0.49)	1.65 (0.44)	1.70 (0.46)	1.14 (0.32)

[a] ARI, *C. arietinum*; RET, *C. reticulatum*; ECH, *C. echinospermum*; JUD, *C. judaicum*; PIN, *C. pinnatifidum*; BIJ, *C. bijugum*; CHO, *C. chorassanicum*; YAM, *C. yamashitae*; CUN, *C. cuneatum*
[b] Standard error in parentheses

these species are predominantly self-pollinated (van der Maesen 1987). Furthermore, outcrossing has been estimated to be less than 1% in the cultivated chickpea (Singh 1987; Tuwafe et al. 1988).

No isozyme polymorphism was detected in 25 accessions of cultivated chickpea accessions originating from six different geographic regions. This is surprising in view of the diverse environments in Asia, the Middle East and several Mediterranean countries, some of which have grown chickpea for at least 7,000 years (van der Maesen 1972), and the presence of abundant genetic variation for other qualitative and quantitative traits (Muehlbauer and Singh 1987). Previously, Oram et al. (1987) studied isozyme variability for 27 isozyme loci in 20 cultivated chickpea accessions and concluded, that as a species, chickpea was relatively poor in genetic variation at isozyme loci. Tuwafe et al. (1988) surveyed isozyme variability in 1,392 accessions of cultivated chickpea from 25 countries, and found polymorphism for only 4 isozyme loci. The predominant alleles at all 4 loci were at a frequency of 0.85 or higher. More recently, Gaur and Slinkard (1990a, b) did not find any genetic variation for isozyme loci in *C. arietinum* and consequently utilized interspecific hybrids of *C. arietinum* with *C. reticulatum* and *C. echinospermum* to study their genetics and linkage. This again indicates the limited variability present at isozyme loci in cultivated chickpea. In the present study only 25 cultivated chickpea accessions were used, and more would have been desirable. Nonetheless, the results obtained here are in general agreement with those obtained by Oram et al. (1987), Tuwafe et al. (1988) and Gaur and Slinkard (1990a, b).

The annual *Cicer* species form four crossability groups (Ahmad 1988). A comparison of the species clustering derived from isozyme-based genetic distances (Fig. 3) and crossability groups indicates a generally good agreement between the two methods. The electrophoretic data suggest that *C. judaicum*, *C. pinnatifidum* and *C. bijugum* are genetically close to each other. Although this relationship is not evident from morphological grounds, crossability studies and cytogenetic characterization of their interspecific hybrids generally attest to this conclusion (Ladizinsky and Adler 1976a, b; Ahmad 1988). *Cicer cuneatum*, which is morphologically very different from *C. arietinum* as well as the other annual *Cicer* species, is also genetically the farthest removed according to the isozyme analysis. There is, however, some discrepancy in the placement of *C. chorassanicum* with *C. yamashitae* in the dendrogram. While *C. chorassanicum* can be hybridized with difficulty to at least two members, *C. judaicum* (Ahmad et al. 1987) and *C. pinnatifidum* (Ahmad 1988), of crossability group II, the genetic distances between them are somewhat high. On the other hand, *C. chorassanicum* is reproductively isolated from *C. yamashitae* and yet placed in the same cluster in the dendrogram. These results suggest that evolution of reproductive barrier(s) does not necessarily follow the divergence of isozyme loci.

Phylogenetic relationships among the nine annual *Cicer* species, based on isozyme analysis (present study) and seed storage protein analysis (Ahmad and Slinkard 1992), agree in general with the electrophoretic data, but show a few inconsistencies. For example, *C. reticulatum* was genetically closer to *C. echinospermum* than *C. arietinum* in the seed protein studies, whereas it was closer to *C. arietinum* in the isozyme studies. Similarly, on the basis of seed protein profile, *C. chorassanicum* was grouped with *C. bijugum*, *C. pinnatifidum* and *C. judaicum*, but was separated from this group and placed together with *C. yamashitae* in the isozyme-based dendrogram. One explanation for the observed inconsistencies in phylogenetic deductions based on different paramaters is that the observations reflect non-constant rates of evolution of proteins in different lineages. Avise and Aquadro (1982) reviewed the literature on "genetic distances" in vertebrates, based on electrophoretic analysis of proteins, and found at least a 20-fold variation among different protein clock calibrations. Discrepancies in proposed phylogenies derived from different parameters have also been reported for *Clarkia* sect. Peripetama (Sytsma and Gottlieb 1986) and also among members of Triticeae (McIntyre 1988). This may also be true for the annual *Cicer* species.

Morphological, geographical, cytological, crossability and seed protein data indicate that *C. arietinum* and *C. reticulatum* are very closely related taxa, leading to the hypothesis that *C. arietinum* was derived from *C. reticulatum* (Ladizinsky and Adler 1975, 1976a, b; Sharma 1983, Ahmad 1988, Ahmad and Slinkard 1992). *Cicer arietinum* and *C. reticulatum* share 19 isozyme alleles and are quite similar to each other as indicated by their high genetic identity (I = 0.79), which is typical of other proposed progenitor-derivative species pairs (Gottlieb 1981). The first essential step in the evolution of annual legumes is the evolution of the annual state from the perennial state. Thus, discovery of the wild annual ancestor of chickpea represents evolution only at the secondary level, and more work will be needed to discover the ancestral perennial species at the primary level. Nevertheless, until that is settled, *C. reticulatum* should be considered the progenitor of the cultivated chickpea.

The rather small genetic distance between *C. arietinum* and *C. echinospermum* indicates that even *C. echinospermum* could have played a role in the evolution of the cultivated chickpea. The probability of this happening, however, is relatively small in light of the apparent sterility of the *C. arietinum* × *C. echinospermum* hybrid (Ladizinsky and Adler 1976a, b) and/or hybrid breakdown as observed in the F_2 (Ahmad 1988).

A comparison of allozyme variability in a number of crop plants with that of their wild progenitors has revealed reduced variability in the cultivated crops. This has been interpreted as a "founder effect" operating in crop domestication (Ladizinsky 1985). This apparently is the case also in chickpea, since many of the isozyme alleles present in *C. reticulatum* were not detected in *C. arietinum*. This may indicate that domestication of chickpea occurred at one place in the Old World and then later spread to other areas of the Fertile Crescent and subsequently to Asia and the Americas.

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