Indian J. Genet., **61**(3): 218-222 (2001)

Phylogenetic relationships of perennial chickpea species *Cicer microphyllum* (Benth.) with its annual relatives as revealed by allozyme polymorphism

Shweta Gargav and P. M. Gaur

Department of Plant Breeding and Genetics, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur 482 004

(Received: January 2001; Revised: May 2001; Accepted: August 2001)

Abstract

Phylogenetic relationships of *Cicer microphyllum* Benth., a perennial chickpea species found in India and Pakistan, were studied with all annual *Cicer* species. The data on allozyme polymorphism were used to calculate Nei's genetic distance between species. An unweighted paired group method with arithmetic averaging (UPGMA) was used to develop phylogenetic tree from the value of Nei's genetic distances. Four phylogenetic groups were identified. The cultivated chickpea (*C. arietinum* L.), its wild progenitor (*C. reticulatum* Lad.) and *C. echinospermum* formed one group, whereas *C. bijugum, C. judaicum* and *C. pinnatifidum* formed another group. The perennial species, *C. microphyllum*, clustered with *C. chorassanicum* and *C. yamashitae* and formed the third group. *C. cuneatum* was the only species in fourth phylogenetic group. *C. microphyllum*, showed largest distance from the species of the phylogenetic group that contains the cultigen suggesting that it may be difficult to cross this species with the cultigen and its closely related species.

Key words : *Cicer microphyllum*, Cicer species, isozyme markers, phylogeny

Introduction

Of the 42 wild species of *Cicer*, only two species, *C. microphyllum* Benth. and *C. songaricum* Steph., both perennial, are found in India [1]. These species are confined to cold arid tract of the Himalayas, Ladakh, and Lahaul and Spiti regions of Himachal Pradesh. Phylogenetic relationships of *C. songaricum* with the annual species of *Cicer* have been reported [2] but no such information is available for *C. microphyllum*. Several useful traits have been identified in *C. microphyllum*. These include deep root system, tolerance to cold and drought and high ovule fertility [3]. Other interesting characteristics of this species are production of 2 to 3 pods/node and 3 to 7 seeds/pod. The information on phylogenetic relationships of *C. microphyllum* with annual *Cicer* species will help in understanding evolutionary path of *Cicer* species and also give an indication of the ease or difficulty with which this species can be crossed with other species.

Data on allozyme polymorphism have been successfully used to establish phylogenetic relationship among species in several genera including the genus *Cicer* [2, 4, 5, 6]. This article describes phylogenetic relationships of *C. microphyllum* with annual species of *Cicer* using data on allozyme polymorphism.

Materials and methods

The chickpea species used in this study included the perennial wild species *Cicer microphyllum*, the annual cultivated species *C. arietinum* and all eight annual wild species (Table 1). Seeds of all wild species were obtained from the ICRISAT, Patancheru, Andhra Pradesh whereas all varieties of the cultivated species were taken from the chickpea germplasm available with the Department of Plant Breeding and Genetics, JNKVV, Jabalpur. Seeds of wild species were scarified then seeds of both wild and cultivated species were treated

Table 1. Accession numbers and origin of wild and cultivated species of chickpea used in this study.

Species	Variety/Accession	Origin
Cicer arietinum	Annegeri, ICCV 2, JG 62, K 850	India
	Amethyst, Barwon, Norwin	Australia
C. bijugum	ICCW 7, ICCW 41	Turkey
C. chorassanicum	ICCW 26	Afghanistan
C. cuneatum	ICCW 47	Ethiopia
C. echinospermum	ICCW 44	Turkey
C. judaicum	ICCW 34	Israel
	ICCW 74	Syria
C. microphyllum	ICCW 128,ICCW 133	Pakistan
C. pinnatifidum	ICCW 11	Turkey
	ICCW 86	Syria
C. reticulatum	ICCW 6, ICCW 8, ICCW 9, ICCW 9A, ICCW45	Turkey
C. yamashitae	ICCW 1, ICCW 2	Afghanistan

with fungicides (2g thiram + 1g carbendazim kg^{-1} seed) and germinated in petriplates. About a week old seedlings were planted in the earthen pots as well as in the field plots. Standard cultural practices were followed to ensure optimum plant growth.

Isozymes of eight enzymes were analysed through starch gel electrophoresis following procedures described by Gaur and Slinkard [7, 8]. The enzymes studied included alcohol dehydrogenase (ADH, E. C. 3.1.3.2), amylase (AMY, E.C. 3.2.1), aspartate aminotransferase (AAT, E.C. 2.6.1.1), esterase (EST, E.C. 3.1.1), formate dehydrogenase (FDH, E.C. 1.2.1.2), glucose phosphate isomerase (GPI, E.C. 5.3.1.9), malate dehydrogenase (MDH, E.C. 1.1.1.37), and peroxidase (PRX, E. C. 1.1.1.1.7). A total of five plants/seeds were taken for isozyme analysis from each accession. The procedure for nomenclature of isozymes and their coding loci was similar to that used by Gaur and Slinkard [7, 8].

Computer programmes (GDID and DENDRO) developed and provided by Dr. Kermit Ritland, Dept. of Botany, University of British Columbia, Vancouver, Canada were used for phylogenetic analysis. The programme GDID (genetic distance/identity and diversity) calculates genetic distances (D) and genetic identities (I) from allele frequencies for all possible pair-wise comparisons of the species following Nei [9]. This programme further calculates Nei's genetic diversity (H_1) and partitions it into within (H_s) and between (D_{st}) species components. The programme DENDRO (dendrogram) uses unweighted pair group method with arithmetic averaging (UPGMA) as suggested by Sneath and Sokal [10] to develop phylogenetic tree (dendrogram) from the value of Nei's genetic distances.

Results and discussion

The eight enzyme systems revealed twelve scorable polymorphic isozyme loci. These were *Adh-1, Adh-2, Amy, Aat-p, Est-1, Est-2, Fdh, Gpi-c, Mdh-1, Prx-1, Prx-2* and *Prx-3*. The monogenic inheritance of the allozymes of these loci has already been reported [7, 8]. Allozymic variations for these loci in nine annual and one perennial species of *Cicer* are given in Table 2.

The interspecific variation was prevalent (Dst = 0.613) while the intraspecific variation was low (H_s = 0.057). The coefficient of gene differentiation was 0.092. The highest proportion of polymorphic loci was found in *C. reticulatum* (0.50) followed by *C. judaicum* (0.42), *C. microphyllum* and *C. pinnatifidum* possessed a low level of polymorphism (0.17). The remaining *Cicer* species did not show any allozyme polymorphism. The highest number of alleles per locus was observed in *C. reticulatum* (1.58) followed by *C. judaicum* (1.42), *C. microphyllum* (1.17) and *C. pinnatifidum* (1.17). The other species had only one allele per locus.

Two of the twelve isozyme loci were found polymorphic in *C. microphyllum*. The level of polymorphism was similar to that observed in *C. pinnatifidum* but lower than that observed in *C. reticulatum* and *C. judaicum*. Though a more reliable inference on the extent of genetic variability present in *C. microphyllum* will require a study of higher number of accessions of this species, the present study gives clear indication that *C. microphyllum* has higher level of genetic variability than the cultivated chickpea.

Seven varieties of the cultigen, varying widely for days to maturity and morphological traits, were studied. These included four Indian and three Australian varieties. None of the isozyme loci was polymorphic in the cultigen. These results further support the previous findings [2, 4, 5, 6, 11, 12, 13, 14] that the cultivated chickpea has a narrow genetic base. On the other hand, *C. reticulatum* (the wild progenitor of the cultivated chickpea), showed the highest level of polymorphism in this study. Several earlier studies [2, 4, 5, 6, 13] have also reported very high levels of allozyme polymorphism in *C. reticulatum*.

Out of the ten species assayed, four species, viz. *C. judaicum, C. microphyllum, C. pinnatifidum* and *C. reticulatum*, showed within species polymorphism at some isozyme loci. In *C. microphyllum, Amy* and *Prx-3* were polymorphic between the two accessions. In *C. judaicum*, polymorphism was observed at the loci *Fdh, Gpi-c, Prx-2* and *Prx-3*. In *C. pinnatifidum Prx-1* and *Prx-2* were polymorphic between the two accessions. Finally, in *C. reticulatum, Amy, Fdh, Gpi-c, Prx-2* and *Prx-3* were polymorphic among the accessions. The remaining six species were monomorphic for all the loci assayed.

The Nei's unbiased genetic distance coefficients (D) and the Nei's genetic identities (I) were calculated for all possible pair wise comparisons among the species and are presented in table 3. The largest genetic distance was observed between *C. judaicum* and *C. yamashitae* (D = 3.061) while, smaller genetic distances were recorded between *C. arietinum* and *C. reticulatum* (D = 0.349), *C. bijugum* and *C. judaicum* (D = 0.353), *C. judaicum* and *C. pinnatifidum* (D = 0.620), *C. reticulatum* and C. echinospermum (D = 0.691), *C. bijugum* and *C. arietinum* (D = 0.693) and *C. bijugum* and *C. echinospermum* (D = 0.693). *C. cuneatum* appeared to be far different from all other *Cicer* species as it has shown very large distances from other species. *C. microphyllum* showed lowest genetic distance with *C. yamashitae* (D = 1.055) and highest with *C. reticulatum* (D = 2.072).

Table 2. Allelic variation for isozyme loci in *Cicer* species

Locus	*C. arietinum*	*C. bijugum*	*C. chorassa-nium*	*C. cuneatum*	*C. echinos-permum*	*C. judaicum*	*C. micro-phyllum*	*C. pinnat-ifidum*	*C. reticu-latum*	*C. yama-shitae*
Adh-1	a	c	c	a	a	c	b	c	a	a
Adh-2	d	d	c	n	d	d	b	d	d/e	a
Amy	c	c	c	e	c	c	c/d	a	c/b	b
Aat-p	e	d	c	f	d	d	b	c	e	a
Est-2	c	d	e	e	a	e	f	b	a	b
Est-3	c	c	e	e	c	a/b	e	a	d	e
Fdh	b	b	b	c	c	a/b	b	b	b/c	b
Gpi-c	b	b	d	a	a	a/b	e	b	b/c	c
Mdh-1	a	a	b	d	a	a	c	a	a	c
Prx-1	d	c	d	c	c	c	c	a/b	d	d
Prx-2	f	c	n	a	g	d/e	i	b/g	g/f	h
Prx-3	d	a	n	c	c	a/b	f/i	g	e/f/g/h/i	n

Table 3. Nei's genetic distances (D) and identities (I) between *Cicer* species

Population		*C. yama-shitae*	*C. bijugum*	C. chora-ssanicum	*C. cuneatum*	*C. echi-nospermum*	*C. judaicum*	*C. micro-phyllum*	*C. pinnati-fidum*	*C. reticulatum*
C. arietinum	D	0.693	1.386	2.485	0.875	0.982	2.036	0.832	0.349	1.792
	I	0.500	0.250	0.083	0.417	0.375	0.131	0.435	0.705	0.167
C. bijugum	D		1.386	2.485	0.693	0.353	1.525	0.650	0.942	2.485
	I		0.250	0.083	0.500	0.702	0.218	0.522	0.390	0.083
C. chorassanicum	D			1.792	2.485	1.115	1.525	1.055	1.422	1.099
	I			0.167	0.083	0.328	0.218	0.348	0.241	0.333
C. cuneatum	D				0.875	1.452	1.748	1.000	2.195	2.485
	I				0.417	0.234	0.174	0.368	0.111	0.083
C. echinospermum	D					0.663	2.036	1.189	0.691	2.485
	I					0.515	0.131	0.305	0.501	0.083
C. judaicum	D						1.631	0.620	1.037	3.061
	I						0.196	0.538	0.355	0.047
C. microphyllum	D							1.992	2.072	1.055
	I							0.136	0.126	0.348
C. pinnatifidum	D								0.830	2.441
	I								0.436	0.087
C. reticulatum	D									1.214
	I									0.297

The phylogenetic relationships of annual *Cicer* species suggested four groups (Fig. 1). The cultigen (*C. arietinum*), its wild progenitor (*C. reticulatum*) and *C. echinospermum* formed one group. *C. microphyllum* showed maximum distance (D > 2.0) from the species of this group. The species *C. bijugum, C. judaicum* and *C. pinnatifidum* were placed in another group. *C. yamashitae* and *C. chorassanicum* were grouped together and *C. microphyllum* found a place in this group. *C. cuneatum* did not cluster with any of the species. Annual species of *Cicer* have been subjected to numerous taxonomic studies. Based on crossability and fertility of hybrids in interspecific crosses, nine annual species of *Cicer* were divided into four crossability groups [15, 16, 17]. *C. arietinum, C. reticulatum* and *C. echinospermum* were placed in first group, *C. bijugum, C. pinnatifidum, C. judaicum* and *C. yamashitae* in the second group, *C. chorassanicum* in the third group and *C. cuneatum* in the fourth group.

Based on crossability and protein banding patterns, *C. reticulatum* was proposed as wild progenitor of the cultivated chickpea [15, 16, 18, 19]. In this study also *C. arietinum* was found closest to *C. reticulatum* (D = 0.349) which further support the above hypothesis on wild progenitor of the cultivated chickpea. The phylogenetic relationships of annual *Cicer* species obtained in the present study are in perfect agreement to those reported earlier from data on allozyme

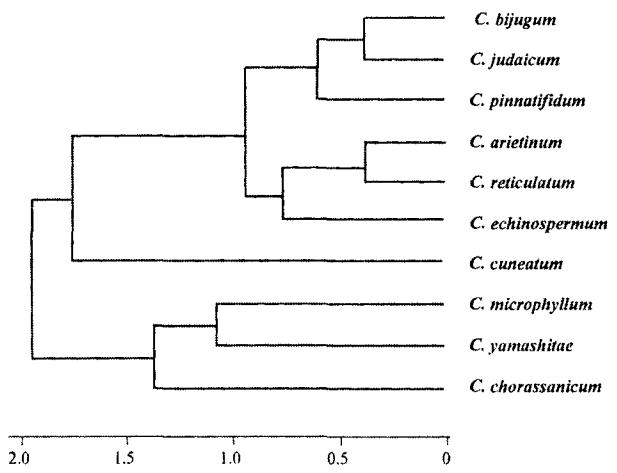

Fig. 1. Dendrogram showing phylogenetic relationships of the perennial species *C. microphylum* with annual species of *Cicer* based on Nei's genetic distances calculated from allozyme polymorphism data

polymorphism [2, 4, 5, 6] seed storage protein banding patterns [20] and RAPD markers [21].

The genetic distances between species showed some correlation with geographic distribution of the wild species. Both the wild species of the first group (*C. reticulatum* and *C. echinospermum*) originated from Turkey. In the second phylogenetic group, both the accessions of *C. bijugum* and one accession of *C. pinnatifidum* originated from Turkey. The other accession of *C. pinnatifidum* and one accession of *C. judaicum* originated from Syria, whereas the remaining one accession of *C. judaicum* originated from Israel. The third phylogenetic group contained the perennial species *C. microphyllum* which originated from Pakistan and two annual species, *C. chorassanicum* and *C. yamashitae*, both of which originated from the neighbouring country Afghanistan. The fourth group contained only one species *C. cuneatum* that originated from Ethiopia.

The phylogenetic relationship of two perennial species, *C. anatolicum* and *C. songaricum*, with annual *Cicer* species have been reported earlier. Kazan and Muehlbauer [4] found that *C. anatolicum* was closest to the species of first phylogenetic group, i.e. the cultivated species, its wild progenitor and *C. echinospermum*. In contrary to this, Tayyar and Waines [2] placed *C. anatolicum* in the third phylogenetic group which contained *C. chorassanicum* and *C. yamashitae*. The other perennial species, *C. songaricum*, was also placed in this group. In the present study, the perennial species *C. microphyllum* was also found in this group. These results suggest that *C. yamashitae* and/or *C. chorassanicum* may be the first annual species evolved from perennial species.

The ultimate objective of studying *C. microphyllum* was to find possibility of its utilization in chickpea improvement programme. Unfortunately, this species failed to flower under normal field conditions. The greater genetic distances of this species with the species of the first phylogenetic group (*C. arietinum*, *C. reticulatum* and *C. echinospermum*) suggest that it may be difficult to cross this species with the cultigen and its closely related species.

Acknowledgement

Supply of the seeds of wild *Cicer* species by the ICRISAT, Patancheru is gratefully acknowledged.

References

1. **Chandel K. P. S.** 1984. A note on the occurrence of wild *Cicer microphyllum* Benth. and its nutrient status. Int. Chickpea Newsl., **10**: 4-5.
2. **Tayyar R. J. and Waines J. C.** 1996. Genetic relationship among annual species of *Cicer* (Fabaceae) using isozyme variation. Theor. Appl. Genet., **92**: 245-254.
3. **Singh B. and Chaurasia O. P.** 1995. Comparative morpho- physiological studies in wild and cultivated gram (*Cicer* spp.) at Ladakh. Adv. Pl. Sci., **8**: 308-311.
4. **Kazan K and Muehlbauer F. J.** 1991. Allozyme variation and phylogeny in annual species of *Cicer* (Leguminosae). Pl. Sys. Evo., **175**: 11-21.
5. **F. Ahmad, Gaur P. M. and Slinkard A. E.** 1992. Isozyme polymorphism and phylogenetic interpretation in the genus *Cicer* L. Theor. Appl. Genet., **83**: 620-627.
6. **Labdi M., Robertson L. D., Singh K. B. and Charrier A.** 1996. Genetic diversity and phylogenetic relationships among the annual *Cicer* species as revealed by isozyme polymorphism. Euphytica, **88**: 181-188.
7. **Gaur P. M. and Slinkard A. E.** 1990. Inheritance and linkage of isozyme coding genes in chickpea. J. Hered., **81**: 455-461.
8. **Gaur P. M. and Slinkard A. E.** 1990. Genetic control and linkage of additional isozyme markers in chickpea. Theor. Appl. Genet., **80**: 648-656.
9. **Nei M.** 1975. Molecular Population Genetics and Evolution. North Holland Publ. Co. Amsterdam.
10. **Sneath P. H. A. and Sokal R. R.** 1973. Numerical Texonomy, W. H. Freeman San Francisco, pp. 100-308.
11. **Oram R. N., Shaikh M. A. Q., Zaman K. M. S. and Brown A. H. D.** 1987. Isozyme similarity and genetic differences in morphology between Hyprosola, a high yielding, high protein mutant of chickpea (*Cicer arietinum* L.) and its parental cultivar. Env. Exp. Bot., **27**: 455-462.
12. **Tuwafe S., Kahler A. L., Boe A. and Ferguson M.** 1988. Inheritance and geographical distribution of allozyme polymorphism in chickpea. J. Hered., **79**: 170-174.
13. **Gaur P. M. and Slinkard A. E.** 1990. New isozyme markers for chickpea. Int. Chickpea Newsl., **23**: 5-8.
14. **Kusmenoglu I., Muehlbauer F. J. and Kazan K.** 1992. Inheritance of isozyme variation in Ascochyta blight resistant chickpea lines. Crop Sci., **32**: 121-127.

15. **Ladizinsky G. and Adler A.** 1976. Genetic relationships among the annual species of *Cicer* L. Theor. Appl. Genet., **48**: 197-293.

16. **Ladizinsky G. and Adler A.** 1976. The origin of chickpea (*Cicer arietinum* L.). Euphytica, **25**: 211-217.

17. **Ladizinsky G., Pickersgill G. B. and Yamamato K.** 1988. Exploitation of wild relatives of the food legumes. *In*: World crops: Cool season food legume (Ed. Summerfield), Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 967-978.

18. **Ladizinsky G.** 1975. A new *Cicer* from Turkey. Notes R. Bot. Gard. Edinburgh, **34**: 201-202.

19. **Ladizinsky G. and Adler A.** 1975. The origin of chickpea as indicated by seed protein electrophoresis. Indian J. Bot., **24**: 183-189.

20. **Ahmad F. and Slinkard A. E.** 1992. Genetic relationships in the genus *Cicer* L as revealed by polyacrylamide gel electrophoresis of seed storage proteins. Theor. Appl. Genet., **84**: 688-692.

21. **Ahmad F.** 1999. Random amplified polymorphic DNA (RAPD) analysis reveals genetic relationship among the annual *Cicer* species. Theor. Appl. Genet., **98**: 657-663.