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Seed protein electrophoresis of some cultivated and wild species of *Chenopodium*

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Abstract

Seed protein profiles of 40 cultivated and wild taxa of *Chenopodium* have been compared by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The relative similarity between various taxa, estimated by Jaccard's similarity index and clustered in UPGMA dendrogram, is generally in accordance with taxonomic position, crossability relationships and other biochemical characters. Eight accessions of *C. quinoa* studied are clustered together and show genetic similarity with closely related *C. bushianum* and *C. berlandieri* subsp. *nuttalliae*. The taxa included under *C. album* complex are clustered in two groups which show that these taxa are a heterogenous assemblage and their taxonomic affinities need a reassessment. Other wild species studied are placed in the dendrogram more or less according to their taxonomic position.

Additional key words: genetic diversity, Jaccard's similarity index, SDS-PAGE, taxonomy, UPGMA dendrogram.

Introduction

The genus *Chenopodium* (*Chenopodiaceae*) includes about 250 species (Giusti 1970) which are mostly colonizing herbaceous annuals occupying large areas in Americas, Asia and Europe, though some are also suffrutescent and arborescent perennials. Economically important species are: *C. quinoa* (2n=36) used as a grain crop, *C. pallidicaule* (2n=18), *C. berlandieri* subsp. *nuttalliae* (2n=36) used for both grain and vegetable and *C. album* (2n=18,36,54) (Mehra and Malik 1963, Heiser and Nelson 1974, Wilson 1980, Mukherjee 1986, Bera and Mukherjee 1987, Pal and Shukla 1990, present study) mainly used as a leafy vegetable and foliage crop, though some Himalayan types are also cultivated for grain (Risi and Galwey 1984, Partap *et al*. 1998).

Phylogenetic relationships between cultivated and their related wild taxa have been studied on the basis of allozyme studies (Wilson 1976, 1981, 1988a,b,c, 1990, Wilson and Heiser 1979, Walters 1987), crossability (Wilson and Heiser 1979, Wilson 1980) and random amplified polymorphic DNA (RAPD) studies (Ruas *et al*. 1999, Gangopadhyay *et al*. 2002). However, full extent of variation within the cultivated types and their relationships with wild species needs to be further elucidated. Moreover, the great morphological, ecological and chromosomal diversity found within *C. album* complex needs further studies to settle the taxonomic problems.

Seed protein electrophoresis has been utilized as a powerful tool in solving taxonomic problems and explaining the origin and evolution of a number of cultivated plants (Ahmad and Slinkard 1992, Jha and Ohri 1996, Nath *et al*. 1997, Vladova *et al*. 2000, El Naggar 2001, Ghafoor *et al*. 2002). The aim of the present study is therefore to study differentiation pattern in seed protein profiles of 40 wild and cultivated taxa of both grain and vegetable chenopods.

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Abbreviations: NTSYS-PC - numerical taxonomy and multivariate analysis system; OTU - operational taxonomic unit; PAGE - polyacrylamide gel electrophoresis; SDS - sodiumdodecyl sulphate; UPGMA - unweighted pair-group method.

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Materials and methods

Details of the plant material used in the present study are given in Table 1. Protein extraction was carried out by homogenizing cotyledons in 0.1 M Tris-HCl buffer (pH 7.5). Samples of supernatant obtained after centrifugation at 17 600 *g* at 4 °C for 20 min were diluted with sample buffer containing 0.0625 M Tris-HCl pH 6.8, 2.5 % sodium dodecyl sulfate (SDS), 5 % 2-mercaptoethanol and 10 % glycerol and heated in boiling water for 5 min prior to being loaded on gel. Content of total protein in the samples were estimated following the method of Lowry *et al*. (1951). Electrophoresis was carried out in the modified discontinuous SDS-PAGE system of Laemmli (1970) using 10 % acrylamide resolving gel (0.375 M Tris-HCl, pH 8.9) and 4 % stack gel (0.125 M Tris-HCl, pH 6.8).The running buffer was Tris-glycine (0.3 % Tris base, 1.44 % glycine and 0.1 % SDS, pH 8.3). Staining of the gels was done in 0.02 % Coomassie Brilliant Blue R-250 containing 55 % methanol and 7 % acetic acid while destaining was done in the same solution but without the dye. The Rf value for each band was computed from the mean of 4 different runs and 2 extractions. The values were used to prepare a data matrix, with rows corresponding to the characters (unique bands; variables) and column to the species and

Table 1. List of *Chenopodium* species and accessions used in the present study.

No.	Taxon	2n	Accession number	Source
1.	*Chenopodium quinoa* Willd.	36	CHEN 71/78	Gatersleben, Germany
2.	*Chenopodium quinoa* Willd.	36	CHEN 67/78	Gatersleben, Germany
3.	*Chenopodium quinoa* Willd.	36	Ames 22158	U.S.D.A.
4.	*Chenopodium quinoa* Willd.	36	CHEN 92/91	Gatersleben, Germany
5.	*Chenopodium quinoa* Willd.	36	PI 433232	U.S.D.A.
6.	*Chenopodium quinoa* Willd.	36	PI 596498	U.S.D.A.
7.	*Chenopodium quinoa* Willd.	36	Ames 13219	U.S.D.A.
8.	*Chenopodium quinoa* Willd.	36	PI 510537	U.S.D.A.
9.	*Chenopodium quinoa* Willd.	36	PI 587173	U.S.D.A.
10.	*Chenopodium quinoa* Willd.	36	PI 478414	U.S.D.A.
11.	*Chenopodium quinoa* Willd.	36	PI 584524	U.S.D.A.
12.	*Chenopodium bushianum* Allen	54	Ames 22376	U.S.D.A.
13.	*Chenopodium berlandieri* ssp. *nuttalliae* (Saff.) Wilson and Heiser	36	PI 568155	U.S.D.A.
14.	*Chenopodium berlandieri* ssp. *nuttalliae* (Saff.) Wilson and Heiser	36	PI 568156	U.S.D.A.
15.	*Chenopodium album* L.	18	Local	Lucknow, India
16.	*Chenopodium album* L. 'Chandanbathua'	18	Local	Lucknow, India
17.	*Chenopodium album* L. NEFA	54	NEFA	NEFA, India
18.	*Chenopodium album* L. IOWA	54	IOWA	IOWA, USA
19.	*Chenopodium album* L.	54	CHEN 85/82	Gatersleben, Germany
20.	*Chenopodium album* L. 'Michigan'	54	PI 605700	U.S.D.A.
21.	*Chenopodium album* L. 'Mexico'	36	Mexico	Mexico
22.	*Chenopodium album* L. 'Siliguri'	18	Siliguri	Siliguri, India
23.	*Chenopodium amaranticolor* (H.J. Coste and A. Reyn.) H.J. Coste and A. Reyn	54	H.P.	Shimla, India
24.	*Chenopodium giganteum* D. Don	54	CHEN 86/85	Gatersleben, Germany
25.	*Chenopodium album* L.	54	CHEN 95/97	Gatersleben, Germany
26.	*Chenopodium opulifolium* Schrad. ex DC.		CHEN 43/96	Gatersleben, Germany
27.	*Chenopodium vulvaria* L.	18	CHEN 46/75	Gatersleben, Germany
28.	*Chenopodium ambrosioides* L.	16	Local	Lucknow, India
29.	*Chenopodium album* L. (6x)	54	Local	Lucknow, India
30.	*Chenopodium ugandae* (Aell.) Aell.	36	CHEN 77/78	Gatersleben, Germany
31.	*Chenopodium aristatum* L.	18	CHEN 51/83	Gatersleben, Germany
32.	*Chenopodium strictum* Roth.	54	CHEN 47/79	Gatersleben, Germany
33.	*Chenopodium murale* L.	18	Local	Lucknow, India
34.	*Chenopodium album* L.	54	CHEN 63/80	Gatersleben, Germany
35.	*Chenopodium botrys* L.	18	CHEN 94/96	Gatersleben, Germany
36.	*Chenopodium album* L. (4x)	36	Chandigarh	Chandigarh, India
37.	*Chenopodium giganteum* D. Don.	54	PI 596372	U.S.D.A.
38.	*Chenopodium hybridum* L.	18	CHEN 55/83	Gatersleben, Germany
39.	*Chenopodium polyspermum* L.	18	CHEN 52/75	Gatersleben, Germany
40.	*Chenopodium foetidum* Lam.	18	CHEN 19/75	Gatersleben, Germany

their accessions in question (operational taxonomic units, *i.e*. OTUs). The scores were 1 for the presence and 0 for the absence of a band. The data file thus written was used as the input for *NTSYS-PC* (Numerical Taxonomy and Multivariate Analysis System), through *SIMQUAL* module (Programme to compute various association coefficients for qualitative data) software to compute Jaccard's similarity index for all possible lines of taxa (OTUs) in question. The similarity matrix thus generated was used to construct a dendrogram by the unweighted pair-group method with arithmetic means (UPGMA) using *SAHN*, another module in *NTSYS-PC* package (Sneath and Sokal 1973).

Results

Seed protein profiles of 40 *Chenopodium* species and their accessions are exactly reproducible. Seventy two unique polypeptide bands were identified in the taxa studied. Maximum number of bands (30) was present in *C. berlandieri* ssp. *nuttalliae* PI 568156 and minimum (9) in *C. polyspermum* CHEN 52/75 (Fig. 1,2,3). The dendrogram divides all the taxa in two main groups which include different sub groups and clusters. The Rf value of the bands ranged from 0.15 - 1.0. In the first group three accessions of *C. quinoa* (CHEN 71/78, CHEN 67/78 and Ames 22158) show very close similarity with each other ranging from 40 to 70.8 %, therefore are joined in a sub-group together (Fig. 4, Table 1). The second sub group comprises different 2x, 4x and 6x cytotypes of *C. album* in addition to *C. amaranticolor*, *C. giganteum*, *C. opulifolium*. *C. album* 'Chandanbathua' (2x) and *C. album* IOWA (6x) show 50 % similarity, *C. album* NEFA (6x) and *C. amaranticolor* (6x) show 55 % similarity while *C. album* CHEN 85/82 (6x) and *C. giganteum* CHEN 86/85 (6x) show 44 % similarity (Fig. 4). In the other cluster *C. album* PI 605700 (6x), *C. album* Mexico (4x) and *C. album* Siliguri (2x) show a similarity ranging from 65.5 to 70.4 % while *C. opulifolium* forms a sister group to these three taxa showing similarity ranging from 41.9 to 55.6 % (Fig. 4). *C. album* CHEN 95/97 (6x) and *C. vulvaria* 46/75 stood distinct from all these species forming two separate clusters which show genetic homology ranging from 14.8 to 41.4 % with the rest of the taxa in the sub group (Fig. 4). *C. ambrosioides* forms a sister group to all the taxa belonging to the first group showing a genetic homology ranging from 9.1 to 18.2 % (Fig. 4).

The second sub group is divided into two sub-groups which include most of the accessions of *C. quinoa* in addition to *C. bushianum* Ames 22376, *C. berlandieri* ssp. *nuttalliae* (PI 568155, PI 568156) and *C. album* local

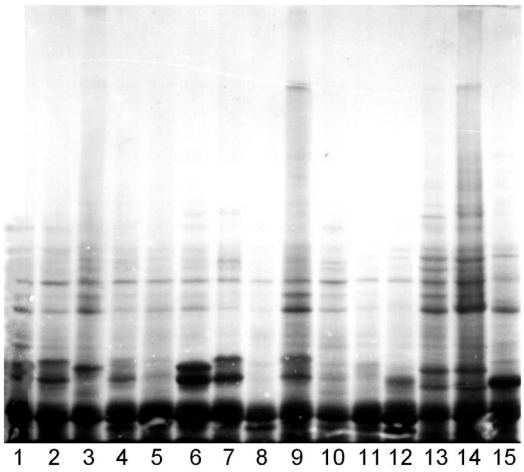

Fig. 1. Electrophoregrams showing seed storage protein banding pattern in *Chenopodium* (serial no. of taxa as as in Table 1).

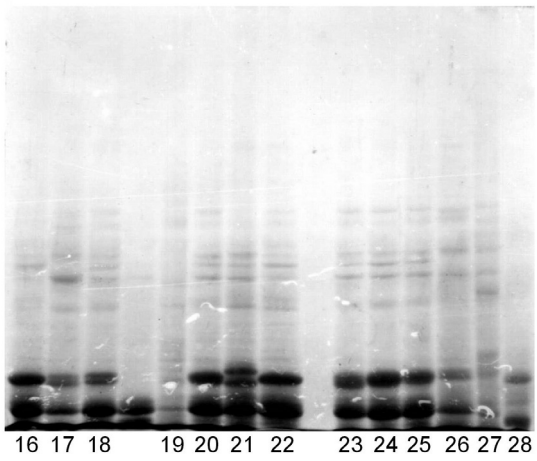

Fig. 2. Electrophoregrams showing seed storage protein banding pattern in *Chenopodium* (serial no. of taxa as in Table 1).

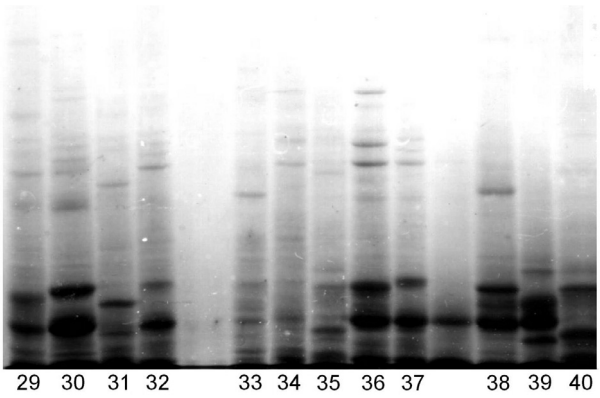

Fig. 3. Electrophoregrams showing seed storage protein banding pattern in *Chenopodium* (serial no. of taxa as in Table 1).

(2x). Eight accessions of *C. quinoa* included in this sub-group show a genetic homology ranging from 53.6 to 70.8 %. Maximum homology of 70.8 % is shown by *C. quinoa* CHEN 92/91 and *C. quinoa* PI 587173. However, the accessions of *C. quinoa* included in the first group *i.e*. *C. quinoa* CHEN 71/78, *C. quinoa* CHEN 67/78 and *C. quinoa* PI 22158 show very low homology with these eight accessions ranging from 2.9 to 14.4 % (Fig. 4). *C. bushianum* Ames 22376 shows very close similarity of 70.8 to 76.2 % with two accessions of *C. quinoa i.e*. *C. quinoa* PI 596498 and *C. quinoa* Ames 13219 (Fig. 4). The overall genetic similarity of *C. bushianum* Ames 22376 with the accessions of *C. quinoa* included in this subgroup ranges from 39.3 to 76.2 %. Two accessions viz. *C. berlandieri* ssp. *nuttalliae* PI 568155 and PI 568156 show a close similarity of 73.3 % while *C. album* local (2x) forms a sister group to these showing a similarity ranging from 43.8 to 44.4 % (Fig. 4). In the other sub group *C. album* local (6x) and *C. strictum* CHEN 47/79 (6x) are joined together with a similarity of 56.5 % (Fig. 4). *C. ugandae* CHEN 77/78 and *C. giganteum* PI 596372 are joined with a 50 % similarity while *C. murale* forms a sister group with similarity ranging from 48.1 % to 50.0 % (Fig. 4).

In the other cluster, *C. album* CHEN 63/80 and *C. album* 'Chandigarh' (4x) are joined with a 50.0 % similarity while *C. aristatum* CHEN 51/83 joins both these with a similarity ranging from 36.7 to 50 % (Fig. 4). The next cluster joins *C. hybridum* CHEN 55/83 and *C. foetidum* CHEN 19/75 with a genetic similarity of 40.9 % (Fig. 4). *C. botrys* CHEN 94/96 and *C. polyspermum* CHEN 52/75 form two separate clusters and show a similarity ranging from 22.2 to 37 % and 19.0 to 33.3 % , respectively with the rest of the taxa in the sub-group (Fig. 4). The heterogeneity among the taxa included in *C. album* complex is clear from the fact that the genetic similarity among them ranges from 6.1 % (*C. album* CHEN 95/97, 6x and *C. album* local, 6x) to 77.8 % (*C. album* Mexico, 4x and *C. album* Siliguri, 2x) (Fig. 4).

Discussion

The taxa presently studied under *C. album* belong to three ploidy levels, diploid (2n=18), tetraploid (2n=36) and hexaploid (2n=54) (Mehra and Malik 1963, Heiser and Nelson 1974, Wilson 1980, Mukherjee 1986, Bera and Mukherjee 1987, Pal and Shukla 1990, Table 1). The heterogenous nature of these taxa is evident from the fact that these are distributed in two main groups in the dendrogram (Fig. 4). In the first group various taxa under *C. album*, *C. amaranticolor*, *C. giganteum* and *C. opulifolium* are joined together forming two sub-groups (Fig. 4) as these all belong to subgroup *Chenopodium* sect. *Chenopodium* subsect. *Chenopodium* (Mosyakin and Clemants 1996). However, *C. vulvaria* CHEN 46/75 and *C. album* CHEN 95/97 (6x), which also belong to subsect. *Chenopodium*, form a sister group to these taxa (Fig. 4). Mosyakin and Clemants (1996) have recommended separation of *C. vulvaria* into an independent subsection which is corroborated by the present study (Fig. 4). *C. ambrosioides* which belongs to entirely different subgenus *Ambrosia* sect. *Ambrina* (Scott 1978) forms a separate subgroup (Fig. 4). This is also supported by studies based on RAPD where two accessions of *C. ambrosioides* form a separate group with a very low genetic similarity to other species (Ruas *et al*. 1999). The *C. album* taxa in the second main group include *C. album* local (2x), *C. album* local (6x), *C. album* CHEN 63/80 and *C. album* 'Chandigarh' (4x). Among these *C. album* local (2x) shows a very close similarity with two accessions of *C. berlandieri* ssp. *nuttalliae* (Fig. 4). This is very well supported by RAPD studies (Ruas *et al*. 1999), however, *C. album* used in this case may not be the diploid *C. album* (2n=18) as studied presently. Here it is worth mentioning that diploid cytotype of *C. album* found in North India is intercrossable with *C. quinoa* and the hexaploid obtained through colchicine treatment of the resultant triploid is fully fertile (M. Pal, personal communication). In the present dendrogram this diploid cytotype of *C. album* also shows close genetic similarity with eight accessions of *C. quinoa* as it is included in the same subgroup (Fig. 4). The hexaploid *C. album* 'local' (2n=54) shows a close similarity with *C. strictum* CHEN 47/79 (2n=54) as both these belong to subgroup *Chenopodium* sect. *Chenopodium* subsect. *Chenopodium* (Mosyakin and Clemants 1996). The tetraploid *C. album* 'Chandigarh' (2n=36) shows close similarity to hexaploid *C. album* CHEN 63/80 (2n=54) while *C. aristatum* CHEN 51/83 belonging to subgroup *Ambrosia* sect. *Botryoides* subsect. *Telexys* (Scott 1978) forms a sister group to these two species. *C. murale* belonging to subgroup *Chenopodium* sect. *Chenopodium* subsect. *undata* (Mosyakin and Clemants 1996) shows close similarity to *C. ugandae* (2n=36) and *C. giganteum* (2n=54) belonging to subgroup *Chenopodium* sect. *Chenopodium* subsect. *Chenopodium* (Mosyakin and Clemants 1996). In fact, *C. murale* has been shown to be one of the progenitors (along with diploid *C. album* types of North India) of the hexaploid (2n=54) *C. album* of North Indian Plains on the basis of seed protein, isozyme, RAPD and tissue culture studies (Gangopadhyay *et al*. 2002). Present study shows the hexaploid *C. album* in the same subgroup as *C. murale*, while the former shows very low genetic similarity with any of the diploid *C. album* types (Fig. 4). This is understandable as 6x and 2x *C. album* types are

not intercrossable (unpublished). The 4x *C. album* growing in Northern India is also very dissimilar with 2x and 6x types. Both 2x and 6x are winter weeds while 4x starts appearing in about mid June and keeps on growing vegetatively till the middle of August, when it starts flowering profusely and the seeds mature in October (unpublished). In the present study the 2x type shows 14.3 % and 20.0 % similarity with 6x and 4x types, respectively, while 6x and 4x types show 35.5 % similarity among them (Fig. 4) which means that 2x is more dissimilar among these cytotypes. The exact taxonomic position and origin of 4x type can be elucidated by further molecular studies.

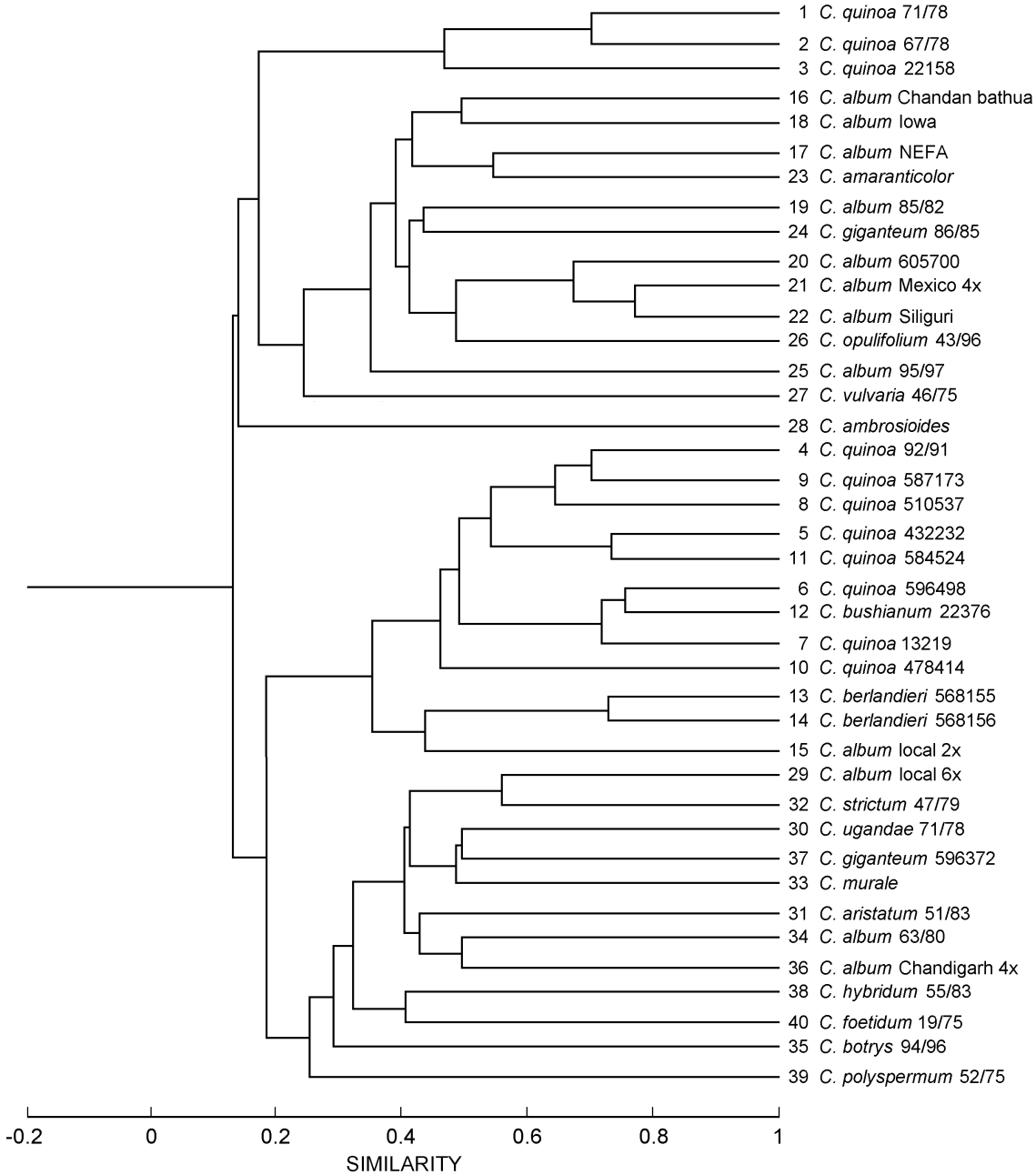

Fig. 4. Dendrogram (UPGMA) of 40 wild and cultivated taxa of *Chenopodium* based on similarity matrix of seed storage proteins (serial number of taxa as in Table 1).

Another remarkable feature of the present study is the separation of *C. quinoa* accessions in the main groups of the dendrogram (Fig. 4). The similarity within the first and second group ranges between 40.0 to 70.0 and 43.8 to 73.9 %, respectively, while these two groups share 2.9 to 14.3 % similarity between them (Fig. 4). Such a sharp

difference within *C. quinoa* accessions in the present study may either be due to some experimental error or due to some inherent differences within *C. quinoa* which has to be sorted out by further detailed studies on seed proteins of large number of *C. quinoa* accessions. In the present discussion we, therefore exclude three accessions of the first group and base the discussion on eight accessions included in the second group which show 43.8 to 73.9 % similarity (Fig. 4). These results are in accordance with those obtained by Wilson (1988a,b) from allozyme variation who showed very low level of allozyme variation in different populations of *C. quinoa* and especially without any differentiation between sympatric domesticated and free-living populations of the Andes. In fact according to Wilson (1990) the origin of *C. quinoa* is monophyletic from Andean crop weed complex through cyclic differentiation and introgressive hybridization. Similar results have been obtained by the electrophoresis data of Lap loci which showed close relationship among the accessions of *C. quinoa* and further confirmed that cultivated and wild taxa of *C. quinoa* are conspecific (Wilson 1976). This has also been corroborated by RAPD studies where 180 markers are shared among all 10 accessions of *C. quinoa* studied with low level of intraspecific differentiation (Ruas *et al*. 1999). *C. bushianum* is included in the same subgroup as eight accessions of *C. quinoa* and shows 39.3 to 76.2 % genetic similarity (Figs. 1,4). This is understandable as *C. bushianum* is included in the same subsect. *Favosa* of the sect. *Chenopodium* as *C. quinoa* (Mosyakin and Clemants 1996). However, the crossability studies show that F1 plants resulting from hybridization between *C. bushianum* and *C. quinoa* are self-sterile although some back cross progeny could be produced (Wilson 1980). *C. bushianum* has been reported to be tetraploid (2n=36) by Wilson (1980) while the material used in the present study is hexaploid (2n=54) (unpublished). Two accessions of *C. berlandieri* ssp. *nuttalliae* also belong to the same subgroup in the dendrogram as *C. quinoa* accessions and *C. bushianum* as these also belong to the subgroup *Chenopodium* sect. *Chenopodium* subsect. *Favosa* (Mosyakin and Clemants 1996). These accessions of *C. berlandieri* ssp. *nuttalliae*, however, show only 26.5 to 64.5 % genetic similarity with eight *C. quinoa* accessions studied (Fig. 4). This is in agreement with independent origin of *C. berlandieri* ssp. *nuttalliae vis-a-vis C. quinoa* as derived from morphological, biochemical and crossability studies (Wilson and Heiser 1979). Crosses between *C. berlandieri subsp. nuttalliae* and *C. quinoa* result in F1 plants with uniformly low fertility while the former shares high level of genomic similarity with its companion weed *C. berlandieri var. sinuatum* (Wilson and Heiser 1979).

C. botrys which belongs to subgroup *Ambrosia* sect. *Botryoides* subsect. *Botrys* (Scott 1978) shows very low level of similarity with the rest of the taxa. Similarly *C. hybridum* belonging to subgroup *Chenopodium* sect. *Grossefoveata* also joins taxa of sect. *Chenopodium* at very low similarity (Fig. 4). Lastly *C. polyspermum* of subgroup *Chenopodium* sect. *Chenopodium* subsect. *Polysperma* (Mosyakin and Clemants 1996) shares only 19.0 and 13.0 % similarity with *C. hybridum* and *C. botrys* respectively (Fig. 4).

It is clear from the above study that seed protein data is congruent with taxonomic position, crossability relationships and other biochemical characters. Further, detailed studies are required to characterize accessions within *C. quinoa* and to elucidate the relationships between various taxa included in *C. album* complex.

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